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**Aortic and mitral valve disease: surgical  
implications of oxidative stress**

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*To my father ..*

*I am extremely proud to have you as my  
father, and my best friend ..*

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# Part I

# 1. Introduction

Aortic and mitral valve diseases are progressively increasing in the Western world, affecting an increasing number of patients. Data from the STS database, the voluntary database that collect data from the vast majority of the USA cardiac surgical centers have shown a steady increase in the surgical procedures done for these two pathologies.



Calendar Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Yearly number of sites in analysis	422	499	549	616	719	801	871	946	980	1,001
Yearly overall procedure count	205,418	227,783	232,044	235,369	250,818	267,861	273,858	287,011	294,384	285,046
<b>Major procedures</b>										
Isolated CABG	146,412	157,324	155,292	151,883	155,015	161,732	164,337	168,040	167,121	158,008
Isolated AVR	9,656	11,342	12,070	12,678	14,946	16,954	18,729	21,379	24,467	25,219
Isolated MVRepl	4,141	4,535	4,176	3,900	4,287	4,498	4,522	4,821	5,326	5,355
AVR + CABG	10,421	12,018	12,437	12,949	13,764	15,539	15,879	17,539	18,807	18,046
MVR + CABG	2,812	3,021	2,815	2,754	2,497	2,648	2,582	2,566	2,578	2,378
AVR + MVRepl	960	1,052	1,071	1,039	1,032	1,163	1,285	1,312	1,490	1,426
Mvrepair	2,755	3,794	3,951	3,977	4,671	5,092	5,424	6,154	6,819	7,207
Mvrepair + CABG	3,171	3,982	4,453	4,310	4,515	4,839	4,854	5,143	4,862	4,635
Not classified above	25,090	30,715	35,779	41,879	50,091	55,395	56,246	60,057	62,914	62,772
<b>Other procedures</b>										
Tricuspid	2,276	3,256	4,086	4,466	5,271	5,965	6,090	6,696	7,139	7,250

From 2001 and 2010, there has been a significant increase for isolated aortic valve replacement (AVR) (from 9656 to 25219 cases) for mitral valve repair (MVRep) (from 2,755 to 7,207 cases), and this is also true for mitral valve replacement (MVR), AVR + CABG, and MVRep + CABG.

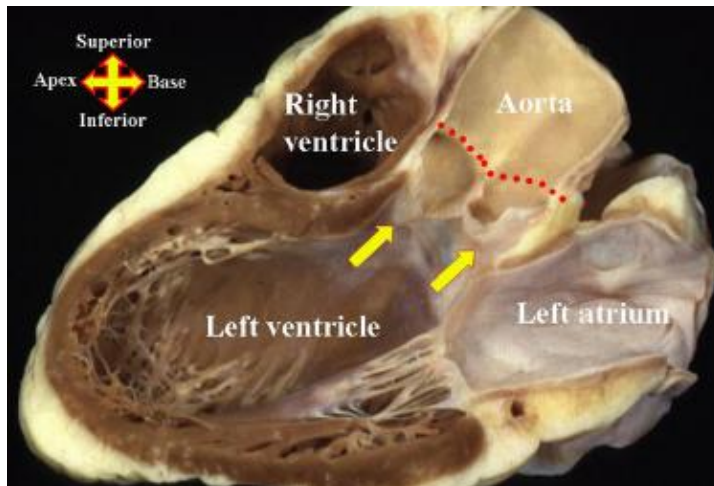
This epidemic of valve disease is due in part to ageing population of Western countries but also to some unknown factors.

In this thesis we will analyze the molecular mechanisms of aortic valve stenosis and mitral valve prolapsed, the possible surgical implications and linking and the role of oxidative stress as a potential main factor in the occurrence and progression of these diseases.

## **2. Aortic valve stenosis: molecular mechanisms and targeted preventive therapies**

### **2.1 Anatomy of the aortic valve**

The aortic valve is a semilunar valve that is quite similar morphologically to the pulmonary valve. Likewise, it does not have a discrete annulus. Because of its central location, the aortic valve is related to each of the cardiac chambers and valves. The aortic valve consists primarily of three semilunar leaflets. As with the pulmonary valve, attachments of the leaflets extend across the ventriculoarterial junction in a curvilinear fashion. Each leaflet therefore has attachments to the aorta and within the left ventricle. The leaflets themselves meet centrally along a line of coaptation, at the center of which is a thickened nodule called the *nodule of Arantius*. Peripherally, adjacent to the commissures, the line of coaptation is thinner and normally may contain small perforations. During systole, the leaflets are thrust upward and away from the center of the aortic lumen, whereas during diastole, they fall passively into the center of the aorta. With normal valvar morphology, all three leaflets meet along lines of coaptation and support the column of blood within the aorta to prevent regurgitation into the ventricle. Behind each leaflet, the aortic wall bulges outward to form the *sinuses of Valsalva*. Two of the three aortic sinuses give rise to coronary arteries, from which arise their designations as right, left, and noncoronary sinuses.

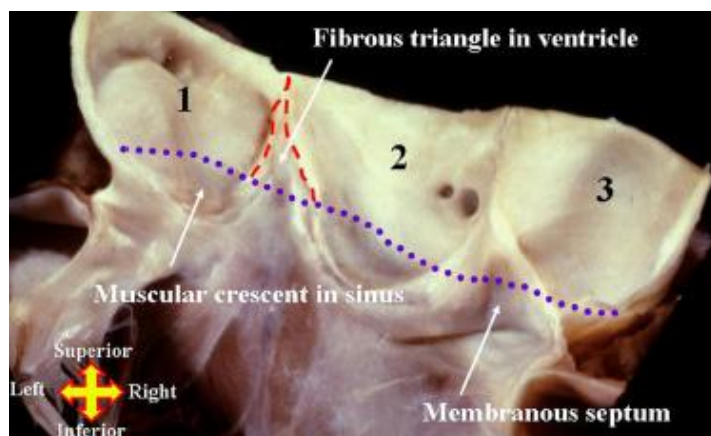


This section through the heart, replicating the parasternal long axis echocardiographic cut, shows how the aortic root is the centrepiece of the heart. The root extends from the basal attachments of the valvar leaflets within the ventricle (yellow arrows) to the sinutubular junction (red dotted line). The compass shows the orientation relative to the remaining thoracic organs.

The *aortic root*, representing the outflow tract from the left ventricle, provides the supporting structures for the leaflets of the aortic valve, and forms the bridge between the left ventricle and the ascending aorta. The root itself, surrounding and supporting the leaflets, has length in that it extends from the basal attachments of the leaflets within the left ventricle to the *sinutubular junction*. The discrete anatomic *ventriculoaortic junction* is a circular locus within this root, formed where the supporting ventricular structures give way to the fibro-elastic walls of the aortic valvar sinuses. This discrete ring, however, is markedly discordant with the morphology of the attachment of the leaflets of the aortic valve. Indeed, it is crossed at several points by the hingelines of the valvar leaflets. These lines, semilunar in structure, extend throughout the root, running from their basal attachments within the left ventricle to their distal attachments at the sinutubular junction. The root as thus defined, therefore, is a cylinder, its walls being made up of the aortic valvar sinuses along with the interdigitating intersinusal fibrous triangles, and with two small crescents of ventricular muscle incorporated at its proximal end. It is the semilunar attachments of the leaflets within the valvar sinuses that form the haemodynamic junction between the left ventricle and the aorta. All structures on the distal side of these attachments are subject to arterial pressures, whereas all parts proximal to the attachments are subjected to ventricular pressures. In functional terms, all three sinuses of the root, and their contained leaflets, are identical. Anatomically, however, it



is necessary to distinguish between the three components. This is best achieved by noting that two of the valvar sinuses give rise to the coronary arteries. These can be nominated as the right and left coronary aortic sinuses. These two sinuses, for their greater part, are made up of the aortic wall. But, because the semilunar attachments of the leaflets cross the anatomic ventriculoaortic junction, a crescent of ventricular musculature is incorporated at the base of each of these two sinuses. The third sinus does not give rise to a coronary artery, and hence, can be designated as the non-coronary aortic sinus. There is no muscular crescent at the base of this sinus, since this sinus has exclusively fibrous walls, the basal part beneath the anatomic ventriculo-aortic junction being part of the important continuity between the leaflets of the aortic and mitral valves that is a feature of the outflow tract of the left ventricle. The areas between the basal attachments of the aortic sinuses within the ventricle itself, which extend distally to the level of the sinutubular junction, are triangular extensions of the left ventricular outflow tract. They are thinned fibrous areas of the aortic wall.

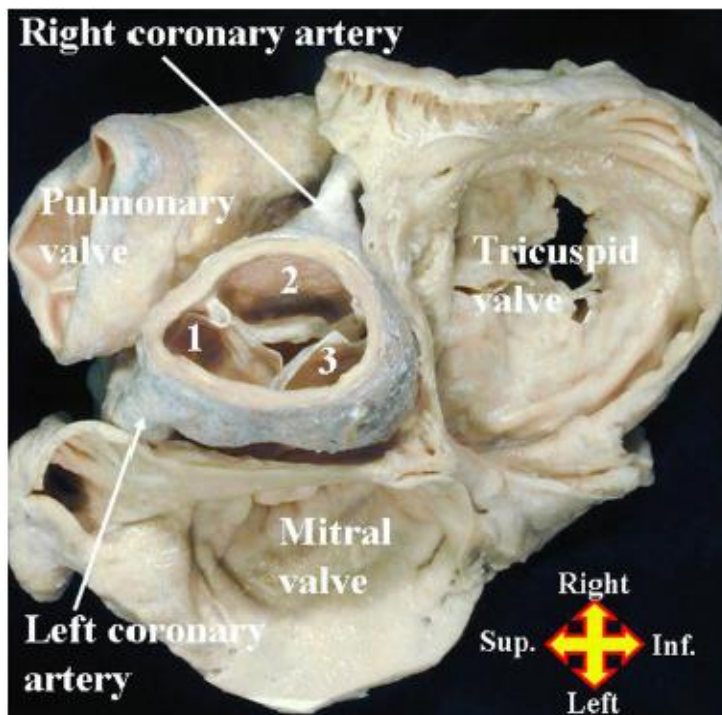


The aortic root has been opened from behind and spread apart, so that the full width of the cylinder can be seen. The aortic valvar leaflets have then been removed, revealing the semilunar nature of their attachments. The purple dotted line shows the anatomic ventriculo-aortic junction, which is the union between the ventricular musculature and the aortic wall at the bases of the left and right coronary aortic valvar sinuses (#1, #2), but between the aortic wall and fibrous continuity with the mitral valve at the base of the non-coronary sinus (#3). Note how the semilunar attachments incorporate muscle at the base of the coronary aortic sinuses, but fibrous tissue within the ventricle as the hingelines extend distally to reach the sinutubular junction (red dashed triangle).

Removing these triangular extensions puts the most distal parts of the left ventricle in direct communication either with the pericardial space or, in the case of the triangle between the two coronary aortic valvar sinuses, with the fibroadipose plane of tissue between the back of the subpulmonary infundibulum and the front of the aorta. The triangle between the left coronary and the noncoronary aortic valvar sinuses is part of the extensive curtain of aortic-to-mitral

valvar fibrous continuity. This triangle, when removed, creates a window to the transverse pericardial sinus, the latter being the space between the back of the aortic root and the anterior atrial walls. The triangle between the non-coronary and the right coronary aortic valvar sinuses is directly continuous with the membranous part of the ventricular septum. The basal part of this fibrous wall is crossed on its right side by the hinge of the tricuspid valve, dividing the membranous septum itself into atrioventricular and interventricular components. The apical part of the triangle extends to the sinutubular junction. Removal of this part creates a window between the left ventricular outflow tract and the right side of the transverse pericardial sinus, opening externally above the attachment of the supraventricular crest of the right ventricle. The third triangle, which separates the two coronary aortic valvar sinuses, is the least extensive of the three. To show the location of this triangle, it is first necessary to remove the free-standing muscular subpulmonary infundibulum. Once this has been done, then it can be seen that removal of the triangle itself creates a window between the subaortic outflow tract and the plane of tissue which separates the aortic root from the infundibulum. The semilunar attachment of the valvar leaflets, therefore, divides the aortic root into supra-valvar and sub-valvar components. The supra-valvar components, the aortic sinuses, are primarily aortic in structure, but contain structures of ventricular origin at their base. The supporting subvalvar parts are primarily ventricular, but extend as thin-walled fibrous triangles to the level of the sinutubular junction. The sinutubular junction itself forms the discrete distal boundary of the root. The valvar leaflets are attached peripherally at this level, and hence, the junction is an integral part of the valvar mechanism. Any significant dilation at the level of the sinutubular junction will produce valvar incompetence. It is moot, therefore, whether stenosis at this level should be labelled as 'supra-aortic', since the sinutubular junction is just as crucial a component of the overall valvar mechanism as are the leaflets and their

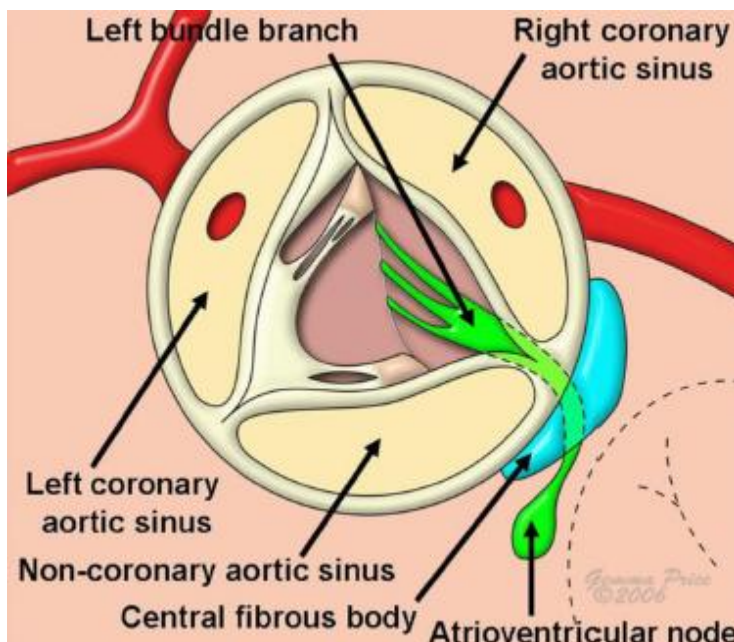
supporting sinuses. Anatomically, the sinutubular junction is no more than the distal extent of the overall valvar complex.



The heart has been dissected by removing the atrial chambers and the arterial trunks, and is photographed from above, looking down on the atrioventricular and ventriculo-arterial junctions. It is orientated as it may be seen by the surgeon. The dissection shows how two of the aortic valvar sinuses (#1, #2) give rise to coronary arteries, and can be nominated as the left and right coronary aortic sinuses, respectively. The third sinus (#3) does not give rise to a coronary artery, and hence, is the non-coronary aortic sinus. Note again that the aortic valve forms the cardiac centrepiece.

When viewed in attitudinally correct orientation the aortic root is positioned to the right and posterior relative to the subpulmonary infundibulum. The *subpulmonary infundibulum* itself is a complete muscular funnel, supporting in uniform fashion the leaflets of the pulmonary valve. The leaflets of the aortic valve, in contrast, are attached only in part to the muscular walls of the left ventricle, since so as to fit the orifices of both aortic and mitral valves within the circular profile of the left ventricle, there is no muscle between them in the ventricular roof. The aortic root, furthermore, is wedged between the orifices of the two atrioventricular valves. The root is related to all four cardiac chambers. These relationships can be well recognised in the clinical situation. The proximity of the root to the anterior interatrial groove is now appreciated by those who have inserted devices via catheters to close defects of the oval fossa, only to find the arms of the devices eroding into the aorta. The relationship to the subpulmonary infundibulum is well demonstrated by the spread of bacterial infection from the valve, or by aneurysmal dilation of the right coronary aortic sinus of Valsalva. The most

important surgical relationship, nonetheless, is probably to the atrioventricular node and the penetrating atrioventricular bundle. The node, located in the wall of the right atrium at the apex of the *triangle of Koch*, is relatively distant from the root. As the conduction axis penetrates through the central fibrous body, however, it is positioned at the base of the interleaflet triangle between the non- and right coronary aortic sinuses. Having penetrated through the fibrous plane providing atrioventricular insulation, the bundle then branches on the crest of the muscular ventricular septum, the left bundle branch fanning out on the smooth left ventricular side, whilst the cord-like right bundle branch penetrates back through the muscular septum, emerging on the septal surface in the environs of the medial papillary muscle. In this position, therefore, the muscular axis responsible for atrioventricular conduction should be relatively distant from most surgical manoeuvres carried out to replace or repair the aortic valve and its supporting structures.



The cartoon shows the location of the atrioventricular conduction axis as it would be seen by the surgeon looking down through the aortic root.

## 2.2 Pathophysiology of aortic valve stenosis

Similarities in risk factors strongly suggest that a similar process underlies both the development and progression of calcific AS as well as atherosclerosis. Risk factors shared by calcific AS and atherosclerosis include hypertension, elevated LDL cholesterol, male gender, smoking, and diabetes mellitus. Epidemiological evidence consistent with these similarities includes the occurrence of valvular heart disease in patients with homozygous familial hypercholesterolaemia, a condition characterised by profoundly elevated LDL cholesterol and premature development of atherosclerosis as well as the connection between aortic valve sclerosis and cardiovascular mortality and morbidity in elderly patients; there is an increased risk of myocardial infarction and death from cardiovascular causes in patients with AS, even among individuals without leftventricular outflow obstruction.

*Palta et al.* investigated the clinical, echocardiographic, and biochemical characteristics that might bear on the rate of progression of AS in a group of 170 consecutive patients who had paired echocardiograms 3 months (23+11) apart. Baseline values were left ventricular outflow tract (LVOT) velocity,  $0.9 \pm 0.2$  m/s; peak aortic velocity,  $2.7 \pm 0.07$  m/s; and aortic valve area (AVA)  $1.17 \pm 0.38$  cm<sup>2</sup>. The annual reduction in AVA was significantly related to the initial AVA ( $r = 0.46$ ,  $P = 0.0001$ ), mean aortic valve gradient ( $r = 20.27$ ,  $P = 0.04$ ), LVOT velocity ( $r = 0.26$ ,  $P = 0.001$ ), and LV end-diastolic diameter ( $r = 0.20$ ,  $P = 0.04$ ) and marginally related to the baseline serum creatinine level ( $r = 0.15$ ,  $P = 0.08$ ). Patients with a serum cholesterol level  $\geq 5.18$  mmol/L (200 mg/dL) had an AVA reduction rate that was about two times as high as that of patients with lower cholesterol levels ( $P = 0.04$ ). The investigators concluded that, in patients with AS, increased levels of serum cholesterol, creatinine, and calcium, as well as current smoking, accelerate the reduction in AVA. Important histopathological evidence that calcific AS unfolds in ways similar to those of atherosclerosis was reported by O'Brien et al.,<sup>15</sup> who found that apolipoproteins B and E are present in early

as well as advanced aortic valve lesions but not in normal valve regions; a similar pattern of lipoprotein deposition also occurs in coronary atherosclerosis. Several studies have helped to explain the process by which aortic valves become calcified. *Mohler et al.* demonstrated that osteopontin, a protein in bone matrix that regulates the deposition of calcium, is found in minimally and severely calcified aortic valves and located mainly in the calcified areas. Additional evidence that aortic valve mineralisation is an active biological process, similar to that occurring in bone, derives from our study of surgically removed valves, which demonstrated an osteoblast phenotype. As shown by reverse transcription–polymerase chain reaction (RT–PCR), increased transcription of genes for osteopontin, bone sialoprotein, osteocalcin, bonespecific transcriptional factor *Cbfa1*, and glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) (i.e. increased mRNA levels) was evident in calcified (vs. normal) aortic valves.

In addition to increased gene transcription, certain signal transduction mechanisms that are upregulated in bone formation also seem to be activated in mineralizing aortic valve leaflets. In particular, LDL receptor-related protein 5 (*Lrp5*), osteocalcin, and other bone markers are upregulated in calcified tricuspid and bicuspid aortic valves. In the presence of hypercholesterolaemia, the *Lrp5* pathway seems to be activated in mesenchymal myofibroblast cells within the valve, switching them to a bone-producing phenotype. This ‘phenotypic switch’ occurs through the binding of *Lrp5* to the glycoprotein Wnt, which in turn activates b-catenin to induce the formation of bone. Upregulation of osteogenic bone signaling markers may be expressed as cartilage formation in human myxomatous (degenerative) mitral valves and calcification in bicuspid and tricuspid valves.

The pathophysiology of calcific AS is no longer considered to be a largely passive process but is rather understood to be an active one, with initiating factors and mechanisms of progression that are broadly similar to those of atherosclerosis in coronary and other arteries. Risk factors

shared by calcific AS and atherosclerosis include hypertension, elevated LDL cholesterol, male gender, smoking, and diabetes mellitus. Calcific AS often develops in persons with atherosclerosis, as indicated (especially in the elderly) by an increased risk of deaths from cardiovascular disease in patients with calcific AS. In addition, calcific AS and atherosclerosis may coexist in persons with homozygous familial hypercholesterolaemia.

Animal studies have shown that, as in atherosclerosis, experimental hypercholesterolaemia initiates endothelial dysfunction in aortic valves, with upregulation of oxidative stress and inflammatory processes leading to plaque formation, as well as a switch to an osteogenic phenotype with valve mineralisation. Evidence for the presence of endothelial dysfunction in the development of calcific AS has been demonstrated in human valves that have been removed surgically. In animal studies of AS, administration of a statin blunted hypercholesterolaemia-induced valve mineralisation and cellular proliferation. This background on AS pathophysiology helps to set the stage for potential benefits of LDL cholesterol-lowering therapy in humans, with the hope that medical therapy may be able to prevent or delay the need for valve replacement. Hypercholesterolaemia may be an important factor in the initiation and progression of AS, and statins may inhibit the induction of this process in animals. If the human aortic valve undergoes a pathophysiological process initiated by hypercholesterolaemia with oxidative modification of cholesterol, as suggested by the studies described earlier, medical therapy might help to decelerate AS progression. The leading indication for surgical valve replacement is calcific AS.

Evidence of endothelial dysfunction in the development of calcific AS in humans has been obtained through the study of surgically excised aortic valves. Appreciation that calcific AS reflects the results of active biological processes has led to the consideration that these processes may be amenable to elimination or slowing by pharmacotherapeutic intervention, potentially avoiding or at least postponing the need for valve replacement.

## REFERENCES

- [1] Wilcox BR, Anderson RH: Surgical Anatomy of the Heart. New York, Raven Press, 1985.
- [2] Sweeney LJ, Rosenquist GC: The normal anatomy of the atrial septum in the human heart. *Am Heart J* 1979; 98:194.
- [3] Manouguian S, Seybold-Epting W: Patch enlargement of the aortic valve ring by extending the aortic incision into the anterior mitral leaflet: New operative technique. *J Thorac Cardiovasc Surg* 1979; 78:402.
- [4] Nicks R, Cartmill T, Bernstein L: Hypoplasia of the aortic root. *Thorax*.
- [5] Oury JH, Angell WW, Eddy AC, Cleveland JC: Pulmonary autograft: Past, present, and future. *J Heart Valve Dis* 1993; 2:365.
- [6] Anderson RH, Becker AE: Cardiac Anatomy. London, *Churchill Livingstone*, 1980.
- [7] Kirklin JW, Barratt-Boyes BG: Anatomy, dimensions, and terminology, in Kirklin JW, Barratt-Boyes BG (eds): Cardiac Surgery, 2d ed. New York, *Churchill Livingstone*, 1993; p 3.
- [8] Schlant RC, Silverman ME: Anatomy of the heart, in Hurst JW, Logue RB, Rachley CE, et al (eds): *The Heart*, 6th ed. New York, McGraw-Hill, 1986; p 16.
- [9] Anderson KR, Ho SY, Anderson RH: The location and vascular supply of the sinus node in the human heart. *Br Heart J* 1979; 41:28.
- [10] Anderson RH: Anatomy of the Aortic Valvar Complex and Its Implications for Transcatheter Implantation of the Aortic Valve. *Circulation* 2008; 74:81.
- [11] Anderson RH: The surgical anatomy of the aortic root. *Multimedia manual of cardiothoracic surgery* 2006; 1:8.
- [12] Cook AC, Anderson RH. Editorial. Attitudinally correct nomenclature. *Heart* 2002;87:503–506.
- [13] Anderson RH. Editorial note: The anatomy of arterial valvar stenosis. *Int J Cardiol* 1990;26:355–360.
- [14] Nalini M. Rajamannan: Update on the pathophysiology of aortic stenosis. *European Heart Journal Supplements* (2008) 10 (Supplement E), E4–E10.



## **2.3 Non-rheumatic calcific aortic stenosis: an overview from basic science to pharmacological prevention**

### **INTRODUCTION**

Calcific aortic valve stenosis is the most common heart valve disease in the Western world, especially in elderly people [1, 2]; on average, 50.000 aortic valve replacements every year occur both in Europe and in the United States due to this pathology, which is the most common valvular disease of the adult [3, 4]. The prevalence of clinically significant aortic stenosis increases progressively with age: it is around 2% in people over 65 [5], and it is more than 4% in octuagenarians [6]. The behavior of aortic valve sclerosis, a milder form of aortic valve disease characterized by calcification and stiffening of the aortic valve without a transvalvular gradient, parallels the one of calcific stenosis, being the prevalence around 20%-30% in patients aged over 65 years [5, 6], and reaching 48%-57% in octuagenarians [6, 7].

This translates in very high costs for the health organizations, that are estimated to be around 1 billion US dollars per year in the United States [1], leading consequently to great interest in medical therapies that could potentially slow the progression of this disease.

In recent years, the assumption that calcific aortic stenosis is a passive, age-related disease has been strongly questioned by studies showing several similarities and some dissimilarities to atherosclerosis in this evolving pathological process and consequently studies investigating the potential role of the most diffuse anti-atherosclerotic drugs, the statins, have been carried out with –unfortunately- controversial results.

In this paper we review the current knowledge on molecular bases of aortic sclerosis and stenosis, the potential preventive therapies, and the results coming from recent clinical trials.

## GENOMICS

Several genomic studies have assessed the possible association between calcific aortic stenosis and genetic factors, concerning mainly atherosclerosis and bone metabolism. About atherosclerosis, even if earlier studies have documented a possible link between lipid metabolism and progression of aortic valve disease [8], studies evaluating the prevalence of apolipoprotein E [9-11], AI [9], e B [9] in patients with aortic stenosis have shown conflicting results. In patients with calcific aortic stenosis there is a prevalence of the allele X+/X+ of apolipoprotein B, but similar allelic frequencies of A alleles [9], whereas it is actually unclear whether apoE allelic variants differ in patients with aortic stenosis from controls; some studies have documented higher prevalence of apoE2 [9] and of apoE4 [11] in aortic stenosis, but these data have not been confirmed by others [10]. Overall, the question whether the frequency of the allelic variants of these lipoproteins is different in aortic stenosis with respect to general population remains still unanswered.

The role of inflammation has also been deeply investigated. Some polymorphisms of the interleuchin-10 gene promoter, namely -1082, -819, -592, are associated to the extent of calcium content of stenotic aortic valves excised during surgical intervention, and the effect of these alleles is further potentiated in patients simultaneously carrying the rare chemokine receptor 5 and connective tissue growth factor alleles [12].

Bone metabolism genomics has been investigated through the assessment of vitamin D receptor genetic polymorphism (BsmI B/b) [13], that predicts bone density or bone mineral mass [14]; patients with aortic valve stenosis show higher frequencies of the B allele, which is associated to reduced calcium adsorption, to more rapid bone loss with advancing age, and to higher parathormone levels [13], suggesting that the bone metabolism profile favoring calcium mobilization from bone could promote aortic valve calcification. In addition, it has been recently shown that a nonsense mutation of the NOTCH1 gene is associated to early

developmental defects and to late de-repression of calcium deposition causing aortic valve disease progression [15]. It is known that NOTCH1 gene plays an important role in hematopoiesis, in stem cell signaling and in cell differentiation during organogenesis, it can also act as a repressor of the transcriptional activity of Runx2 which is important for osteoblast activity, and its inhibition may cause a de-repression of calcium deposition in the aortic valve [15]. Preliminary evidence also suggests a possible role in aortic valve calcification process for PvuII polymorphism of the alpha estrogen receptor, possibly linked to the prevalence of aortic valve calcification in post-menopausal women [16], likely though an increase of cholesterol [16].

Finally, some cell cycle regulatory genes may contribute to calcific aortic disease progression, as it has been shown that the expression of p21<sup>WAF1/CIP1</sup> (cyclin-dependent protein-kinase inhibitor p21), and of 14-3-3  $\sigma$  genes is reduced, both at a transcriptional and translational level, in calcific aortic valves [17]. This points to cell cycle control as an attractive potential target for future therapies aimed at reducing the progression of aortic valve disease, although evidence up to date suggests that only bone metabolism polymorphisms have a definite role in the progression of aortic valve disease and the role of lipid metabolism genomics and of cell cycle regulation is still at the hypothesis level.

## ATHEROSCLEROSIS

The development and progression of calcific aortic stenosis is an active atheroinflammatory process consisting of local inflammatory components and deposition of plasma lipoproteins in the lesions; this process shares several features with atherosclerosis with –however- some important differences.

Initial lesions of aortic valve disease are quite similar to atherosclerosis by nature and include disruption of the basement membrane, subendothelial accumulation of intracellular lipids and lipoproteins, and infiltration of foam cells, nonfoam cells, and T lymphocytes, together with

local and systemic activation of inflammation [2, 18]. On the other hand, calcification is more extensive in aortic valve disease than in atherosclerosis, and fibrocalcific thickening is responsible for the clinical manifestations of the disease [19, 20]. In this section we resume the studies which have addressed the possible relation between aortic valve disease (sclerosis and stenosis) and atherosclerosis.

**Endothelial function.** Patients with aortic valve sclerosis have impaired endothelium-dependent, post-ischemic, flow-mediated dilation [21]; in addition, aortic valve sclerosis is associated with higher intima-media thickness, a marker of early atherosclerotic vascular structural changes [22], with respect to normal subjects [23]. This further confirms and extends the current concept that relates aortic valve sclerosis, atherosclerosis, and increased risk of future cardiovascular events for patients affected by valve sclerosis [24-27].

Endothelial damage in patients with calcific aortic valve disease is further supported by the demonstration of increased E-selectin plasma levels in patients affected by severe aortic stenosis which return to normal after surgery [28]. The evidence showing higher circulating levels of endothelial microparticles that are correlated with the number of activated monocytes [29], provides another link between aortic disease and atherosclerosis. Finally, studies performed on aortic valve specimens collected at intervention time have shown that diseased aortic valves express more markedly several endothelial markers such as CD31, CD34, von Willebrand factor, and CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule), with respect to normal valves [30], further supporting the association between endothelial dysfunction and calcific aortic valve disease progression.

**Lipid metabolism.** Among traditional risk factors for atherosclerosis, lipid metabolism abnormalities have been frequently associated with calcific aortic valve disease. In 1994 Otto et al. described the possible links between lipid metabolism and calcific aortic disease

showing, in diseased aortic valves, the presence of large amounts of intracellular and extracellular neutral lipids that could not be found in normal valves [27]; since then, hyperlipemia has been widely investigated as a possible mechanism underlying aortic valve stenosis development and progression, and several studies support a strong association between lipid metabolism and aortic valve disease. It has been recently shown that plant sterols accumulate in stenotic aortic valves in a direct relation to their respective serum concentrations [31]; this is a proof of concept that lipid metabolism is strictly related to the development and progression of aortic valve disease, as it clearly demonstrates that circulating lipids are capable of entering in aortic valve leaflets and thus to exert local effects in the interstitium of aortic valves. In addition, angiotensin converting enzyme co-localizes with circulating LDL and apolipoprotein B in sclerotic and stenotic aortic valves [32], suggesting that angiotensin converting enzyme may be concentrated in aortic lesions through the retention of plasma lipoproteins. Moreover, stenotic aortic valves contain increased levels of low-density lipoprotein receptor-related protein-5 (LRP5) [33], a member of the low-density lipoprotein receptor-related proteins family of cell-surface receptors that are involved in diverse biologic processes, including lipid metabolism, retinoid uptake, and neuronal migration; LRP5 plays an important role in the activation of skeletal bone development and in the differentiation process of aortic valve mesenchymal myofibroblasts into osteoblasts, providing another link between lipid metabolism and aortic valve calcification [33].

Additional evidence suggesting a strong relation among lipid metabolism, atherosclerosis and aortic valve disease comes from the post-mortem analysis of the aortic valve of a 7-years old boy who died in the 50's because of type IIb familial hypercholesterolemia; even in this young boy, atherosclerotic lesions with plaques rich in lipid-laden foam cells, focal areas of collagen and scant basophilic round substances were found in aortic valve tissue[34].

From a clinical standpoint, studies have also addressed the potential role of atherosclerosis risk factors in the development and progression of calcific aortic stenosis, showing that high LDL and lipoprotein (a) levels are risk factors both for aortic valve sclerosis [6, 35, 36] and for stenosis as well [37], even if recent studies limit that effect to patients less than 65 years old [38]. In addition, it has also been shown that metabolic syndrome is associated with aortic stenosis progression over time and this can be explained in great part by the fact that patients affected by metabolic syndrome had greater LDL cholesterol levels than controls (124 mg/dL vs. 85 mg/dL) [39]. Hypercholesterolemia as a causative factor for aortic valve disease is not a new concept, as in 1997 Wilmschurst et al showed that patients suffering from aortic valve stenosis had higher total cholesterol levels [8]; more recently, Pohle et al have documented that hypercholesterolemic subjects (LDL > 130 mg/dL) have much greater progression of aortic valve calcifications over time, with an average annual increase of calcium of 43% vs. an increase of 9% in controls ( $p < 0.001$ ) [40]. Besides LDL, the overall lipid profile seems to play a role in aortic valve disease, as not only high LDL levels but also high total cholesterol, low HDL levels, and an higher total cholesterol/HDL ratio are independently associated to higher progression rates [41]; also particle size affects progression of aortic valve disease, since small circulating LDL particles (< 255 Angstrom) are increased in patients with faster disease progression [42]. This could be related to greater accumulation of oxidized LDL in aortic valve tissue favoring fibrocalcific remodeling of the aortic valve, providing additional arguments that support the hypothesis that aortic stenosis is a lipid-driven process [43]. Finally, Busseuil et al. have shown that, in rabbits, the infusion of an ApoA-1 mimetic peptide leads to the regression of cholesterol-induced aortic valve stenosis, suggesting alternative molecular targets for medical treatment of this disease [44].

**Inflammation.** From more than a decade it is well known that aortic valve “early lesions” are characterized by inflammatory features including T lymphocytes and macrophages [27],

but the contribution of inflammation and its mediators has recently become more detailed as described by growing evidence. Several inflammation mediators accumulate in diseased aortic valves: the terminal complement complex C5b-9 is found in sclerotic and even more in stenotic aortic valves [45], that also show increased expression of C3a and C5a receptors [45]; in addition, stenotic aortic valves show increased expression of interleukin-1 $\beta$  and of leukocyte infiltrates [46], of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) [47] (and this could promote calcification through apoptosis induction of the interstitial aortic cells [48]), of extracellular matrix tumor necrosis factor-  $\alpha$  (TNF- $\alpha$ ) [49], and of endothelial adhesion molecules intercellular adhesion molecule-1 (ICAM-1) [28, 50, 51], of vascular cell adhesion molecule-1 (VCAM-1) [28, 51], of Heat Shock Protein-60 [51], of vascular adhesion protein-1, eotaxin-3 and of monokine induced by interferon-gamma [47]; such a variety of inflammation mediators strongly suggests a sensible contribution of inflammation in the course of aortic valve disease.

Of note is also the fact that normal aortic valves have a substantial expression of the Toll-like receptors 2 e 4, which are known to play an important role in pro-inflammatory cytokine expression in several cell population, especially in interstitial cells [52]; in vitro studies have also shown that Toll-like receptors are fully functional in interstitial aortic valve cells, as peptidoglycan or lipopolysaccharide stimulation can cause NF- $\kappa$ B pathway activation with consequent production and release of proinflammatory cytokines, e.g. interleukin-6 - IL-8 - ICAM-1, and of the osteogenesis-related factors bone morphogenetic protein-2 and Runx2 [52], supporting a causative link between inflammation and osteogenesis in aortic valve disease. Such a finding is further supported by evidence concerning the pathway osteoprotegerine/ RANK/RANKL [53, 54] that will be wider discussed in the osteogenesis section.

**Haemostasis.** Aortic valve stenosis has been for long time associated with bleeding disorders; in 1958 Heyde described the association between frequent gastrointestinal bleeding episodes and aortic valve stenosis (Heyde's syndrome) [55]; later, it has been reported that the bleeding in context of aortic stenosis is due to acquired type 2A von Willebrand disease, characterized by a decrease of the large multimers of the von Willebrand factor due to the action of ADAMTS-13, a matrix metalloproteinase that acts on von Willebrand factor preferentially under conditions of high shear stress (e.g. stenotic aortic valves) [56, 57]. This phenomenon leads in some cases to clinically significant bleeding episodes [58, 59], such as recurrent gastrointestinal bleedings sometimes associated to angiodysplasia [55, 58, 59]; of note, bleeding episodes can sometimes be controlled only by aortic replacement, when von Willebrand factor multimers return to normal levels [55, 58-60]. This perturbation of von Willebrand factor multimers also affects platelet function with a reduction in platelet count [56], prolonged bleeding time [60], impaired shear-induced platelet aggregation [56], prolonged closure time at platelet function analyzer [57], and lower P-selectin levels with respect to controls [61]. Usually, von Willebrand factor levels and multimers return to normal after within six months after surgery [56, 57, 59], with the exclusion of patient-prosthesis mismatch [57, 58], although not all studies agree upon this. In fact, Yoshida et al., using immunoblotting electrophoresis, have shown a direct linear relation between von Willebrand factor antigen and the indexed effective orifice area of the valve prosthesis [59]; on the other hand, Goldsmith and coll., using ELISA, have documented an inverse relation between plasma von Willebrand factor and the size of the aortic prosthesis [61]. In summary, evidence concerning von Willebrand factor behavior before and after surgery for aortic valve disease, as well as the differences in the return to baseline in patients with or without patients-prosthesis mismatch suggest that the abnormalities related to this molecule are not causative of aortic valve disease but much more likely its consequences.



Fewer informations are –unfortunately– available about other haemostatic variables; preoperative fibrinogen levels are higher in patients candidates to aortic valve replacement with respect to controls [61], and this could be due to an increased pro-inflammatory status. In addition, patients affected by aortic or mitral disease have a more marked activation of coagulation and fibrinolysis, being prothrombin factor F1.2, thrombin-antithrombin complex and d-dimer higher than in controls, even if it is actually not known whether aortic and mitral valve disease differ somehow in this aspect [62]. Moreover, hypertensive patients who also have aortic valve sclerosis features show higher levels of prothrombin factor F1.2 with respect to hypertensive patients without aortic valve sclerosis [63]. Finally, experimental evidence on experimental animals suggests that an atherogenic diet (cholesterol-rich or cholesterol-rich plus the addition of vitamin D) promotes the development of aortic valve sclerosis together with tissue factor expression on the aortic side of the leaflets [64].

In summary, current knowledge concerning aortic valve disease and coagulation, fibrinolysis, and platelet activation markers suggest that also these pathways can be involved in aortic valve disease progression, but additional evidence is necessary to prove these potential associations.

**Angiogenesis.** Angiogenesis is defined as the outgrowth of new vessels from pre-existing blood vessels [65], is regulated by a balance between angiogenic activators such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 and platelet-derived growth factor and inhibitors [66], and is one of the main mechanisms involved in atherogenesis promoting plaque growth, intraplaque hemorrhage, and lesion instability [67, 68]. Several studies have shown that neoangiogenesis occurs in the course of calcific aortic disease, and a substantial association with inflammatory infiltrates [30, 51, 69, 70] occurs; interestingly, vascular density is higher in aortic valves with a low or intermediate grade of calcification and lower or even null in severely stenotic valves [69, 70], suggesting that neoangiogenesis

has a specific temporal pattern in the development of this disease. Neovascularization is also associated with increased expression of VEGF and of its receptors Flt-1 e Flk-1, of endothelial nitric oxide synthase [69], and of SPARC (secreted protein, acidic and rich in cysteine/osteonectin) [70], an extracellular matrix protein contributing to embryonic development, blood vessels formation, and tissue remodeling [71]. Further proof concerning angiogenesis role in calcific aortic disease comes also from ex vivo studies showing that new capillary sprouts exhibit endothelial markers as CD31, von Willebrand factor, CD34, and tyrosine kinase receptor Tie-2 on their surface. Finally, Chalajour et al. have identified, in diseased valves, the presence of a cell population expressing both endothelial (VEGF receptor-2 and tyrosine kinase receptor Tie-2 expression) and mesenchimal (smooth muscle alpha-actin expression) markers and with an enhanced angiogenic activity that could promote the pathological angiogenesis occurring in stenotic valves via the differentiation of these cells into the cell types needed for this process [72]. Taken together, these data suggest that angiogenesis is one of the player in the field of calcific aortic stenosis, being a potentially new therapeutic target to slow the progression of this disease.

**Extracellular matrix remodeling.** Parallel to neovessels formation, diseased aortic valves are place of intense remodeling of the extracellular matrix favoring disease progression. Several matrix metalloproteases (MMPs), a family of zinc-dependent endopeptidases that degrade extracellular matrix [73] are increasingly expressed in stenotic aortic valves with respect to controls. In particular, an increase in MMP-1 [46, 49, 74], that co-localizes in aortic valves with TNF- $\alpha$  has been observed, which suggests an important link between matrix remodeling and inflammation [49]. In addition, other MMPs have been assessed in this pathological condition, as MMP-3 [74, 75], MMP-9 [74-77]. Concerning MMP-2, even if some studies have provided evidence for a potential involvement of this MMP in the development of aortic valve disease, other have not confirmed this finding [74,

76, 78-80]. The behavior of tissue inhibitors of MMPs 1 and 2 (TIMPs) is even more controversial: some studies failed to show TIMPs parallel increases [49] or have shown minor increases [77], whereas others have documented sensible increases of these inhibitors [75, 80]; it is thus possible that the balance among MMPs and their inhibitors in stenotic aortic valves is dependent from cycles or phases of disease progression that are not still well characterized, where the prevalence of tissue remodeling may vary over time. In addition, other enzymes with lytic actions on elastic fibers are much more represented in stenotic aortic valves than in normal valves: cathepsin G levels are higher in mast cells of patients affected by aortic valve stenosis [81]; cathepsins S, K e V are also increased, cathepsin V especially in endothelial cells and cathepsin S more in the calcified portions of the valves [82]; moreover, the association between cathepsin G and TGF-1 $\beta$  in mast cells further supports the main role of inflammation activation in the global process [81]. The inhibition of these proteases, as well as the modulation of mast cell activation and of their release of lytic substances is a new potential therapeutic target that could help lowering aortic valve disease progression [83]; and this is particularly meaningful as it has been recently hypothesized that activated mast cells located in the subendothelium of human coronary arteries with parietal microthrombi might contribute to endothelial damage and erosion releasing proteolytic substances that damage endothelial cells attachment to subendothelium [84].

**Oxidative stress.** It has been documented that, in stenotic aortic valves, oxidative stress is increased, as shown by higher levels of superoxide and of hydrogen peroxide compared with controls in the calcified and peri-calcific regions of the valve [85, 86]. Parallel to the increase in reactive oxygen species, there is also a reduction in the expression and activity of antioxidant enzymes catalase and of NADPH oxidase nitric oxide scavenger (Nox) Nox2 and Nox4 [85]. This is somehow an unexpected finding, as it is well known that upregulation of Nox signaling is an important contributor to atherosclerosis and vascular remodeling [87].

Unlike atherosclerosis, oxidative stress increase occurring in calcific aortic valve disease is not due to NADPH oxidase activity rise which, in stenotic valves, is reduced because of “NOS uncoupling”, when molecular oxygen, rather than arginine, becomes the terminal electron recipient with generation of superoxide radicals by nitric oxide synthase [85]. The NADPH oxidase downregulation in stenotic aortic valves has not been confirmed, however, by others [86], leaving still open the question concerning the mechanisms causing increased oxidative stress in aortic valve disease and if these differ or not from the ones active in atherosclerosis.

**Infection.** The hypothesis that an infective agent, *Chlamydia Pneumoniae*, was related to development and progression of the aortic valve stenosis, has been pursued by several Authors over the years, sharing with the infective hypothesis of atherosclerosis the same fortune, a mix of success and failures [88-90]. Both for aortic sclerosis [91] and for aortic valve stenosis [92-96], studies addressing the potential association between *Chlamydia* and calcific aortic disease have not provided definitive answers. About the possible role of other infectious agents, Bratos-Perez et al. have recently shown that a significant percentage of severely stenotic aortic valves collected at surgery (48/75, 64%), once cultured in appropriate media, show the presence of growing self-replicating calcifying nanoparticles, also called nanobacteria, that potentially represent new pathogens under scrutiny, whose presence has already been found in carotid disease, abdominal aorta aneurysms, and in calcific human vessels or valves [97]. Nevertheless, further evidence is needed for a clear demonstration of the role of these agents in calcific aortic disease.

## BONE METABOLISM

The role of calcium metabolism in calcific aortic stenosis has been always considered definite since for several decades calcific aortic stenosis has been associated with diseases

characterized by perturbations in calcium metabolism such as chronic renal failure and hemodialysis [98, 99]. This role has then been confirmed by several papers demonstrating a possible direct association with ionized calcium [100] and an inverse one with total serum calcium [101]; in addition, aortic stenosis has been related with higher serum parathormone and lower vitamin D levels in male patients affected by aortic stenosis and coronary disease [102]. These data, together with evidence concerning an higher frequency of the B allele of Vitamin D in patients with aortic stenosis (see “Genomics” section), confirm the importance of systemic calcium metabolism, especially in terms of increased calcium mobilization, in the progression of this disease.

In addition, even if the concept of calcification has been always related to non-rheumatic aortic stenosis (which has been frequently called “calcific”) the process underlying calcification and, eventually, bone has been attributed for nearly a century to a passive degenerative process.

Only recently, several studies have documented that calcium deposition and bone formation in stenotic aortic valves is an actively regulated process and that the aortic valve specimens collected during aortic valve replacements show heterotopic ossification, active bone remodeling and mature lamellar bone [103]; in addition, in these valves there are increased levels of several osteoblast markers such as osteopontin [85, 104, 105], osteocalcin [104], osteoprotegerin [105], bone sialoprotein [104, 105], and of the osteoblast-specific transcription factor Cbfa1 [85, 104]. Moreover, the endochondral process of bone formation leading to aortic valve calcification seems to share several features with mature bone [33], and is strictly associated with inflammation [103, 106, 107] and neoangiogenesis [30, 51, 103]; besides, the pathway Lrp5/Wnt, osteoblast differentiation signaling markers that play a primary role in bone development [33], and the osteoprotegerin/RANK (receptor activator of nuclear factor- $\kappa$ B/RANKL (the RANK receptor) axis are also involved in aortic valve

calcification [53, 54, 108]. RANKL, a member of TNF- $\alpha$  superfamily, is a transmembrane protein expressed on osteoblasts surface, stromal cells, T cells and endothelial cells, that interacts with the transmembrane protein RANK on osteoclasts precursors or on mature osteoclasts promoting their differentiation via NF- $\kappa$ B. This interaction can be effectively blocked by circulating osteoprotegerin that is able to stop osteoclasts differentiation [109, 110]. Both in aortic sclerosis [54] and in aortic stenosis [53, 54, 111] the relative concentrations of the components of this axis are sensibly changed with respect to controls, with increases in RANK e RANKL and a decrease in osteoprotegerin, a pattern promoting aortic valve calcification. Current data suggest that the process of aortic valve calcification is thus a multifactorial event where several pathways converge to enhance disease progression, a complex mechanism that might request a multi-target approach for its modulation. The recent demonstration that also adrenergic and purinergic pathways can contribute to this process, confirms and expands the concept of the need of multiple target modulation. Osman et al. have in fact shown that  $\beta$ -adrenergic receptors, especially  $\beta_2$  ones, are upregulated in stenotic valves, mainly in peri-calcific areas [111], and the prolonged incubation of cultured interstitial aortic valve cells with a stable analogue of the purinergic receptor P2Y (ATP- $\gamma$ -S) causes their differentiation into osteoblasts [112], further supporting a multi-effector and multi-cause process.

## FLUID MECHANICS

In addition to the role of genomics, atherosclerosis and bone metabolism, fluid mechanics perturbations that progressively occur at the site of aortic stenosis may themselves contribute to the progression of the disease amplifying the biological changes that underlie the evolution of aortic stenosis. As blood velocity increases through a stenotic aortic valve, local endothelial shear stress changes and –ultimately- turbulent flow occur. This can activate

endothelial cells, tipping the balance of endothelial-derived factors to disrupt barrier function, and enhance coagulation, leukocyte adhesion, and smooth muscle cell proliferation [113]. Even if these responses likely exist as protective mechanisms, these changes may render these responses excessive, resulting in damaged tissue, impaired organ function, and an abnormal fibroproliferative response. Besides the previously described effects on hemostasis via von Willebrand factor perturbations, local shear stress changes can affect several different pathways related with the progression of aortic stenosis; among them cell cycle, nitric oxide and prostacyclin release, oxidative stress, lipoprotein uptake, synthesis and permeability, inflammation, vascular muscle cell migration, differentiation and proliferation, and neoangiogenesis [114-117]. Finally, it is interesting to note that different shear stress patterns can determine the endothelial and smooth muscle cell phenotype towards activation or quiescence [118, 119], further supporting the role of fluid mechanics in aortic stenosis.

### **Pharmacologic modulation of calcific aortic stenosis progression: basic mechanisms and clinical results**

From the previous sections it is clear that several mechanisms involved in atherosclerosis are also possibly involved in the development and progression of calcific aortic stenosis; the class of drugs more widely investigated, on a experimental ground and in clinical practice, are, as a consequence, the HMG-CoA reductase inhibitors, named statins. It is well known that statins exert significant anti-inflammatory effects and that they also preserve endothelial function in the general population [120, 121], and this concept this might be translated into potential beneficial effects for patients affected by aortic valve disease [1, 19, 122], although a definitive demonstration of the “aortic protection” is unfortunately still lacking. Atorvastatin reduces the progression of aortic valve calcification in rabbits fed with an atherogenic diet via Lrp5 pathway [123] and endothelial nitric oxide synthase [124] modulation; in vitro studies have also shown that atorvastatin also reduces the activity of alkaline phosphatase, a marker

of osteoblast activity, in cultured interstitial aortic cells previously incubated in osteogenic media obtained with the addition of compounds with purinergic activity [112]. Other studies have assessed the role of statin treatment on different cell populations of the aortic valve showing that, in animal cell cultures, these drugs exert their maximal effect by limiting the formation of calcific nodules and alkaline phosphatase activity of aortic myofibroblasts via downregulation of HMG-CoA reductase activity; on the other hand, in osteoblasts, statins favor alkaline phosphatase activity, and they also promote the differentiation in osteoblasts of other cell populations such as bone marrow stromal cells [125]. This effect, called “statin paradox”, suggests the hypothesis that timing of statins administration may be critical in the reducing disease progression, as the prevailing cell populations during the various phases of the development of aortic stenosis may differ, as well as the response to drug treatment. Other potentially beneficial biological effects of statins include the attenuation in the expression of pro-inflammatory mediators, although this modulation is incomplete. Indeed, statins reduce the expression of the inflammatory markers eotaxin3, and of a monokine induced by interferon-gamma, but not of TGF- $\beta$  and of vascular adhesion protein-1 [47]. Another proof of paradoxical biological effects of statins is the demonstration that these drugs reduce of the expression of regulators of G protein-mediated signaling (RGS) proteins RGS2, RGS3 and RGS4 in stenotic aortic valves leading to increase in the activation of extracellular-regulated kinases [126] that in turn stimulate further proliferation of myofibroblasts; this suggest that, even in this setting, not all the effects of statins are potentially beneficial for prevention of stenotic aortic disease progression. This is not totally unexpected, due to the complex pleiotropic activities of statins on the overall isoprenoid pathway. Indeed, inhibition of the HMG-CoA reductase enzyme by statins, results in the inhibition not only of cholesterol biosynthesis, but it influences the generation of a variety of isoprenoid compounds, important in cell cycle, inflammation and osteogenesis, as well.



Concerning clinical studies, both retrospective and prospective studies have addressed the question whether statin therapy can reduce the progression of this disease, reaching controversial results, as the majority of retrospective studies document a protective effect of statins on aortic valve stenosis evolution, whereas the majority of prospective and all prospective and randomized trials do not. The prospective randomized SALTIRE has enrolled patients affected by aortic stenosis with total cholesterol levels  $\geq 150$  mg/dL and with a jet velocity  $\geq 2.5$  m/sec; overall, patients average jet velocity was 3.42 m/sec, with a peak gradient of 48 mmHg, and an aortic valve area of  $1.03 \text{ cm}^2$ . The results of SALTIRE show that a high-dose (80 mg/die) of atorvastatin therapy fails to show any effect, at a median follow-up of 25 months, on the primary end-points of the study, assessing aortic stenosis progression. The echocardiographic annual change in jet velocity was 0.199 m/s in atorvastatin-treated patients, and 0.203 m/s in placebo-treated ( $p=0.95$ ), whereas the valve calcium score increase, assessed with helical computed tomography, was 22.3% and 21.7% in statin and placebo group, respectively ( $p=0.93$ ) [127]. Unlike SALTIRE trial, the RAAVE trial, a prospective open-label study assessing the effect of rosuvastatin treatment on aortic stenosis over 18 months, has documented significant improvements in aortic stenosis progression in statin-treated patients. With respect to SALTIRE, patients enrolled in RAAVE had higher aortic valve areas ( $1.23 \text{ cm}^2$ ) but similar jet velocities (3.63 m/s) at baseline; in this latter study, treatment was not assigned by randomization, but patients presenting with abnormally high LDL cholesterol levels ( $> 130$  mg/dL) received rosuvastatin, whereas patients with lower LDL levels received no statin. RAAVE has shown that aortic valve area decreased annually in both groups, but statin-treated patients had half the progression with respect to untreated patients (decrease in aortic valve area  $0.05$  vs.  $0.1 \text{ cm}^2$  per year,  $p=0.04$ ) [128].

Recently, results of the prospective randomized study SEAS, the biggest one up to date in this field, have been published; in this study 1873 patients with LDL values < 236 mg/dL, with an average aortic valve area of 1.28 cm<sup>2</sup> and an average jet velocity of 3.1 m/s, were enrolled; these patients have been randomized to be treated for a minimum of four years with placebo or ezetimibe/simvastatin, for a median follow-up of 52.2 months. In SEAS, ezetimibe/simvastatin was no better than placebo in reducing the primary composite end point of aortic valve-related and cardiovascular events (defined as death from cardiovascular causes, aortic-valve replacement, congestive heart failure as a result of progression of aortic-valve stenosis, nonfatal myocardial infarction, hospitalization for unstable angina, coronary-artery bypass grafting, percutaneous coronary intervention, or non-hemorrhagic stroke), occurring similarly in treated patients (35.3%) and in controls (38.2%, p=0.59). In addition, the secondary end-point of aortic valve-related events (defined as aortic-valve replacement surgery, congestive heart failure due to aortic stenosis, or death from cardiovascular causes) did not differ between groups, occurring in 32.6% and 35.1% of cases and of controls, respectively (p=0.73). On the other hand, this treatment was significantly more effective than placebo in reducing the risk of ischemic events, a secondary composite end point driven primarily by reductions in coronary artery bypass graft surgery; this occurred in 15.7% and 20.1% of cases and of controls, respectively (p=0.02) [129]. In addition, this study has raised some concerns over the significantly increased risk of cancer of treated patients being doubled in patients who received ezetimibe/simvastatin. The new cancers, however, were not specific to one site, and an interim analysis of ongoing ezetimibe/simvastatin studies SHARP and IMPROVE-IT, plus the analysis from SEAS, revealed no increased risk [130].

Finally, the results of another randomized trial, ASTRONOMER, are expected for the end of this year; this study will address the effect of rosuvastatin (40 mg/d) treatment on aortic valve stenosis progression in patients in 272 patients with an average jet velocity of 3.2 m/s, an

aortic valve area of  $1.2 \text{ cm}^2$ , a mean transvalvular gradient of 22 mmHg, half of them having bicuspid aortic valve, for a 3 to 5 years follow-up period [131]. In addition, other prospective randomized studies concerning statins and aortic valve disease are currently running, and the results of a search of these trials on the site [www.clinicaltrials.gov](http://www.clinicaltrials.gov), performed on 26/08/2008), is reported in Table 1.

As stated before, evidence from retrospective nonrandomized studies is in contrast with what has been previously shown by randomized ones. Although publication bias in this case can not totally be excluded, data from these studies consistently show that statin therapy reduces the progression of aortic valve disease, whatever the degree of stenosis was documented at the beginning of follow-up, and this result has been documented both with echocardiography [132-135] or with CT scan for detection of calcium content of the aortic valve [40, 136, 137]. Based on these data, we can infer that statins are a class of drugs with potentially beneficial effect on the reduction of valve disease progression, but this is not demonstrated yet; it is also possible, in our opinion, that timing is the most critical factor for the optimal efficacy of this class of drugs, and that their maximal effects are obtainable at earlier stages of the disease such as aortic sclerosis. In support of this hypothesis, a recent retrospective study of Antonini-Canterin analyzing 1046 patients over 19 years has shown that, at an average follow-up of  $5.6 \pm 3.2$  years, statins can reduce aortic stenosis progression only in the milder degrees of the disease, such as in case of aortic sclerosis (jet velocity  $\geq 1.5$  and  $< 2.0$  m/s) or in case of moderate aortic stenosis (jet velocity  $\geq 2$  and  $< 3$  m/s). When aortic stenosis was more severe (jet velocity  $\geq 3$  and  $< 4$  m/s), the effect of statins was null [138]. If this will be confirmed by appropriate studies, it may strongly impact prevention programs of health systems which will have to consider to screen for and to treat aortic sclerosis. It should be noted, however, that all the randomized trials done up to date or still running might have unfortunately missed the patient population where statins are more effective, as all studies have enrolled patients with

jet velocities almost always  $> 3.0$  m/s, and therefore might fail to provide evidence of the potential benefits of statins in the relatively early phases of aortic valve disease, namely during the phase of aortic sclerosis. Finally, it is well known that the progression of sclerosis and stenosis and especially of sclerosis toward stenosis is substantially unpredictable in the individual patient, being in some cases very slow, in others quite accelerated, and that different patterns of aortic valve disease progression (linear and non-linear) are possible [139]; this, together with evidence concerning the potential beneficial effects of statins in aortic valve sclerosis and not in aortic valve stenosis, suggests that the assumption that valve sclerosis is always and invariably the precursor of valve stenosis may not be completely correct.

Converting enzyme inhibitors are another class of drugs with potentially beneficial effects on aortic stenosis progression as the presence of converting enzyme has been shown both in sclerotic [18, 32] and stenotic aortic valves [32, 140], as well as chimase [140]; both these enzymes are involved in Angiotensin II production, a molecule with proinflammatory and profibrotic activity; but clinical data, although scarce, actually do not show any effect of these drugs on this disease [135].

Moreover, preliminary evidence from animal models suggest that olmesartan, an angiotensin type 1 receptor antagonist, reduces atherosclerotic changes in aortic valves by preserving endothelial cells integrity and inhibiting transdifferentiation into myofibroblasts or into osteoblasts in valve leaflets [141], but no clinical data about this class of drugs is still actually available.

Finally, another potential therapeutic target to reduce aortic stenosis progression is smoke cessation. It has been shown that nicotine and acetaldehyde, both smoke components, induce TGF- $\beta$ 1 expression in cultured fibroblasts, and nicotine can also activate mast cells; both these mechanisms can ultimately lead to an increased collagen/elastin ratio in valve tissue

[81]; this finding suggests a potential role also for smoke cessation in the prevention of calcific aortic disease.

## CONCLUSIONS

The progression of calcific aortic stenosis is a multi-factorial process resembling the atherosclerotic process, with nevertheless some important differences in the final results achieved, since in atherosclerosis the final result is plaque development and plaque instability whereas calcific aortic disease ends up in severe calcification of the aortic valve. This suggests that calcific aortic stenosis is not a single disease process but it seems to be more likely a common macroscopic anatomic equivalent of a series of partly related processes that ultimately lead to severe calcification of the valve.

The multitude of the mechanisms potentially involved in aortic disease progression, together with the available clinical evidence strongly suggests that drug therapy aimed at reducing this progression will have necessarily to be multi-factorial and will have to address the earliest stages of the disease, as it is now clear that drug therapy administered in more advanced stages of the disease may be ineffective or, at best, much less effective. The time has come to integrate all the possible mechanisms affecting the development and progression of aortic stenosis into a unique effort to discover innovative therapies that may lead to lower evolution over time.

Table 1  
Ongoing studies available on [clinicaltrials.gov](http://clinicaltrials.gov) (26/08/2008) concerning aortic valve stenosis and statin therapy

Study	Institution (Principal investigator), patients	Treatment	Duration of follow-up	Primary end-point	Secondary end-point	Expected termination
AORTICA	AORTICA GROUP (Pedro L Sanchez); 164 patients with jet velocity > 2 m/sec	Placebo, Fluvastatin (80 mg)	12 months	Changes in C-reactive protein concentration at the end of treatment	Changes in inflammatory markers, in valve area, and in transvalvular gradients at the end of treatment	November 2008
ROCK-AS	Helsinki Univ. (Markku Kupari); 120 patients candiudates to surgery	Placebo, Rosuvastatin (20 mg), Candesartan (16 mg), Candesartan +Rosuvastatin	3-5 months	Inflammation degree in stenotic aortic valves explanted during the intervention	Calcification, lipid accumulation and fibrosis degree in stenotic aortic valves explanted during the intervention	December 2013
STAAT	Leipzig Univ. (Claudia Walther); 100 patients with 1.5 > aortic valve area >0.8 cm <sup>2</sup>	Placebo, Fluvastatin (20-40 mg)	24 months	Aortic valve stenosis progression assessed as valve area, peak and mean transvalvular gradients at echocardiogram, and as peak-to-peak gradient at catheterization	Not available	December 2008
STOP-AS	Cleveland Clinic (Brian P Griffin); 70 patients with 1.7 > aortic valve area >0.9 cm <sup>2</sup>	Atorvastatin (40 mg) (vs internal controls and vs historical controls)	24 months	Rate of change in the aortic valve area as measured by transthoracic echocardiography	Rate of change in the aortic valve area measured by transthoracic echocardiography compared to that of historical controls, to standard of care group, and change in the mean and peak gradients across the aortic valve as measured by transthoracic echocardiography in the treated group compared to historical control group	June 2008 (not yet published)

## REFERENCES

- [1] Moura LM, Maganti K, Puthumana JJ, Rocha-Goncalves F, Rajamannan NM. New understanding about calcific aortic stenosis and opportunities for pharmacologic intervention. *Curr Opin Cardiol*. 2007;22:572-7.
- [2] Rajamannan NM, Otto CM. Targeted therapy to prevent progression of calcific aortic stenosis. *Circulation*. 2004;110:1180-2.
- [3] Iung B, Baron G, Butchart EG, Delahaye F, Gohlke-Barwolf C, Levang OW, Tornos P, Vanoverschelde JL, Vermeer F, Boersma E, Ravaud P, Vahanian A. A prospective survey of patients with valvular heart disease in Europe: The Euro Heart Survey on Valvular Heart Disease. *Eur Heart J*. 2003;24:1231-43.
- [4] Rajamannan NM. Calcific aortic stenosis: medical and surgical management in the elderly. *Curr Treat Options Cardiovasc Med*. 2005;7:437-42.
- [5] Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *N Engl J Med*. 1999;341:142-7.
- [6] Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, Kitzman DW, Otto CM. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol*. 1997;29:630-4.
- [7] Lindroos M, Kupari M, Heikkilä J, Tilvis R. Prevalence of aortic valve abnormalities in the elderly: an echocardiographic study of a random population sample. *J Am Coll Cardiol*. 1993;21:1220-5.
- [8] Wilmshurst PT, Stevenson RN, Griffiths H, Lord JR. A case-control investigation of the relation between hyperlipidaemia and calcific aortic valve stenosis. *Heart*. 1997;78:475-9.
- [9] Avakian SD, Annicchino-Bizzacchi JM, Grinberg M, Ramires JA, Mansura AP. Apolipoproteins AI, B, and E polymorphisms in severe aortic valve stenosis. *Clin Genet*. 2001;60:381-4.
- [10] Ortlepp JR, Pillich M, Mevissen V, Krantz C, Kimmel M, Autschbach R, Langebartels G, Erdmann J, Hoffmann R, Zerres K. APOE alleles are not associated with calcific aortic stenosis. *Heart*. 2006;92:1463-6.
- [11] Novaro GM, Sachar R, Pearce GL, Sprecher DL, Griffin BP. Association between apolipoprotein E alleles and calcific valvular heart disease. *Circulation*. 2003;108:1804-8.
- [12] Ortlepp JR, Schmitz F, Mevissen V, Weiss S, Huster J, Dronskowski R, Langebartels G, Autschbach R, Zerres K, Weber C, Hanrath P, Hoffmann R. The amount of calcium-deficient hexagonal hydroxyapatite in aortic valves is influenced by gender and associated with genetic polymorphisms in patients with severe calcific aortic stenosis. *Eur Heart J*. 2004;25:514-22.
- [13] Ortlepp JR, Hoffmann R, Ohme F, Lauscher J, Bleckmann F, Hanrath P. The vitamin D receptor genotype predisposes to the development of calcific aortic valve stenosis. *Heart*. 2001;85:635-8.
- [14] Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. *Nature*. 1994;367:284-7.
- [15] Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D. Mutations in NOTCH1 cause aortic valve disease. *Nature*. 2005;437:270-4.
- [16] Nordstrom P, Glader CA, Dahlen G, Birgander LS, Lorentzon R, Waldenstrom A, Lorentzon M. Oestrogen receptor alpha gene polymorphism is related to aortic valve sclerosis in postmenopausal women. *J Intern Med*. 2003;254:140-6.

- [17] Golubnitschaja O, Yeghiazaryan K, Skowasch D, Schild H, Bauriedel G. p21WAF1/CIP1 and 14-3-3 sigma gene expression in degenerated aortic valves: a link between cell cycle checkpoints and calcification. *Amino Acids*. 2006;31:309-16.
- [18] O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. *Arterioscler Thromb Vasc Biol*. 1996;16:523-32.
- [19] Rashidi A, Adler DS, Casscells W, Madjid M. Is it time to prescribe statins to patients with calcified aortic stenosis? *Am Heart J*. 2005;150:41-5.
- [20] Helske S, Kupari M, Lindstedt KA, Kovanen PT. Aortic valve stenosis: an active atheroinflammatory process. *Curr Opin Lipidol*. 2007;18:483-91.
- [21] Poggianti E, Venneri L, Chubuchny V, Jambrik Z, Baroncini LA, Picano E. Aortic valve sclerosis is associated with systemic endothelial dysfunction. *J Am Coll Cardiol*. 2003;41:136-41.
- [22] Baldassarre D, De Jong A, Amato M, Werba JP, Castelnuovo S, Frigerio B, Veglia F, Tremoli E, Sirtori CR. Carotid intima-media thickness and markers of inflammation, endothelial damage and hemostasis. *Ann Med*. 2008;40:21-44.
- [23] Yamaura Y, Nishida T, Watanabe N, Akasaka T, Yoshida K. Relation of aortic valve sclerosis to carotid artery intima-media thickening in healthy subjects. *Am J Cardiol*. 2004;94:837-9.
- [24] Branch KR, O'Brien KD, Otto CM. Aortic valve sclerosis as a marker of active atherosclerosis. *Curr Cardiol Rep*. 2002;4:111-7.
- [25] Faggiano P, Antonini-Canterin F, Erlicher A, Romeo C, Cervesato E, Pavan D, Piazza R, Huang G, Nicolosi GL. Progression of aortic valve sclerosis to aortic stenosis. *Am J Cardiol*. 2003;91:99-101.
- [26] Novaro GM, Griffin BP. Calcific aortic stenosis: another face of atherosclerosis? *Cleve Clin J Med*. 2003;70:471-7.
- [27] Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation*. 1994;90:844-53.
- [28] Ghaisas NK, Foley JB, O'Brien DS, Crean P, Kelleher D, Walsh M. Adhesion molecules in nonrheumatic aortic valve disease: endothelial expression, serum levels and effects of valve replacement. *J Am Coll Cardiol*. 2000;36:2257-62.
- [29] Diehl P, Nagy F, Sossong V, Helbing T, Beyersdorf F, Olschewski M, Bode C, Moser M. Increased levels of circulating microparticles in patients with severe aortic valve stenosis. *Thromb Haemost*. 2008;99:711-9.
- [30] Chalajour F, Treede H, Ebrahimnejad A, Lauke H, Reichensperner H, Ergun S. Angiogenic activation of valvular endothelial cells in aortic valve stenosis. *Exp Cell Res*. 2004;298:455-64.
- [31] Helske S, Miettinen T, Gylling H, Mayranpaa M, Lommi J, Turto H, Werkkala K, Kupari M, Kovanen PT. Accumulation of cholesterol precursors and plant sterols in human stenotic aortic valves. *J Lipid Res*. 2008;49:1511-8.
- [32] O'Brien KD, Shavelle DM, Caulfield MT, McDonald TO, Olin-Lewis K, Otto CM, Probstfield JL. Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma. *Circulation*. 2002;106:2224-30.
- [33] Caira FC, Stock SR, Gleason TG, McGee EC, Huang J, Bonow RO, Spelsberg TC, McCarthy PM, Rahimtoola SH, Rajamannan NM. Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. *J Am Coll Cardiol*. 2006;47:1707-12.
- [34] Rajamannan NM, Edwards WD, Spelsberg TC. Hypercholesterolemic aortic-valve disease. *N Engl J Med*. 2003;349:717-8.



- [35] Palta S, Pai AM, Gill KS, Pai RG. New insights into the progression of aortic stenosis: implications for secondary prevention. *Circulation*. 2000;101:2497-502.
- [36] Peltier M, Trojette F, Sarano ME, Grigioni F, Slama MA, Tribouilloy CM. Relation between cardiovascular risk factors and nonrheumatic severe calcific aortic stenosis among patients with a three-cuspid aortic valve. *Am J Cardiol*. 2003;91:97-9.
- [37] Gerber Y, Goldbourt U, Feinberg MS, Segev S, Harats D. Are triglyceride-rich lipoproteins associated with aortic valve sclerosis? A preliminary report. *Atherosclerosis*. 2003;170:301-5.
- [38] Owens DS, Katz R, Johnson E, Shavelle DM, Probstfield JL, Takasu J, Crouse JR, Carr JJ, Kronmal R, Budoff MJ, O'Brien KD. Interaction of age with lipoproteins as predictors of aortic valve calcification in the multi-ethnic study of atherosclerosis. *Arch Intern Med*. 2008;168:1200-7.
- [39] Briand M, Lemieux I, Dumesnil JG, Mathieu P, Cartier A, Despres JP, Arsenault M, Couet J, Pibarot P. Metabolic syndrome negatively influences disease progression and prognosis in aortic stenosis. *J Am Coll Cardiol*. 2006;47:2229-36.
- [40] Pohle K, Maffert R, Ropers D, Moshage W, Stilianakis N, Daniel WG, Achenbach S. Progression of aortic valve calcification: association with coronary atherosclerosis and cardiovascular risk factors. *Circulation*. 2001;104:1927-32.
- [41] Yilmaz MB, Guray U, Guray Y, Cihan G, Caldir V, Cay S, Kisacik HL, Korkmaz S. Lipid profile of patients with aortic stenosis might be predictive of rate of progression. *Am Heart J*. 2004;147:915-8.
- [42] Mohty D, Pibarot P, Despres JP, Cote C, Arsenault B, Cartier A, Cosnay P, Couture C, Mathieu P. Association between plasma LDL particle size, valvular accumulation of oxidized LDL, and inflammation in patients with aortic stenosis. *Arterioscler Thromb Vasc Biol*. 2008;28:187-93.
- [43] Cote C, Pibarot P, Despres JP, Mohty D, Cartier A, Arsenault BJ, Couture C, Mathieu P. Association between circulating oxidised low-density lipoprotein and fibrocalcific remodelling of the aortic valve in aortic stenosis. *Heart*. 2008;94:1175-80.
- [44] Busseuil D, Shi Y, Mecteau M, Brand G, Kernalleguen AE, Thorin E, Latour JG, Rheume E, Tardif JC. Regression of aortic valve stenosis by ApoA-I mimetic peptide infusions in rabbits. *Br J Pharmacol*. 2008;154:765-73.
- [45] Helske S, Oksjoki R, Lindstedt KA, Lommi J, Turto H, Werkkala K, Kupari M, Kovanen PT. Complement system is activated in stenotic aortic valves. *Atherosclerosis*. 2008;196:190-200.
- [46] Kaden JJ, Dempfle CE, Grobholz R, Tran HT, Kilic R, Sarikoc A, Brueckmann M, Vahl C, Hagl S, Haase KK, Borggreffe M. Interleukin-1 beta promotes matrix metalloproteinase expression and cell proliferation in calcific aortic valve stenosis. *Atherosclerosis*. 2003;170:205-11.
- [47] Anger T, Pohle FK, Kandler L, Barthel T, Ensminger SM, Fischlein T, Weyand M, Stumpf C, Daniel WG, Garlisch CD. VAP-1, Eotaxin3 and MIG as potential atherosclerotic triggers of severe calcified and stenotic human aortic valves: effects of statins. *Exp Mol Pathol*. 2007;83:435-42.
- [48] Jian B, Narula N, Li QY, Mohler ER, 3rd, Levy RJ. Progression of aortic valve stenosis: TGF-beta1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. *Ann Thorac Surg*. 2003;75:457-65; discussion 65-6.
- [49] Kaden JJ, Dempfle CE, Grobholz R, Fischer CS, Vocke DC, Kilic R, Sarikoc A, Pinol R, Hagl S, Lang S, Brueckmann M, Borggreffe M. Inflammatory regulation of extracellular matrix remodeling in calcific aortic valve stenosis. *Cardiovasc Pathol*. 2005;14:80-7.

- [50] Mazzone A, Epistolato MC, Gianetti J, Castagnini M, Sassi C, Ceravolo R, Bevilacqua S, Glauber M, Biagini A, Tanganelli P. Biological features (inflammation and neoangiogenesis) and atherosclerotic risk factors in carotid plaques and calcified aortic valve stenosis: two different sites of the same disease? *Am J Clin Pathol.* 2006;126:494-502.
- [51] Mazzone A, Epistolato MC, De Caterina R, Storti S, Vittorini S, Sbrana S, Gianetti J, Bevilacqua S, Glauber M, Biagini A, Tanganelli P. Neoangiogenesis, T-lymphocyte infiltration, and heat shock protein-60 are biological hallmarks of an immunomediated inflammatory process in end-stage calcified aortic valve stenosis. *J Am Coll Cardiol.* 2004;43:1670-6.
- [52] Meng X, Ao L, Song Y, Babu A, Yang X, Wang M, Weyant MJ, Dinarello CA, Cleveland JC, Jr., Fullerton DA. Expression of functional Toll-like receptors 2 and 4 in human aortic valve interstitial cells: potential roles in aortic valve inflammation and stenosis. *Am J Physiol Cell Physiol.* 2008;294:C29-35.
- [53] Kaden JJ, Bickelhaupt S, Grobholz R, Haase KK, Sarikoc A, Kilic R, Brueckmann M, Lang S, Zahn I, Vahl C, Hagl S, Dempfle CE, Borggrefe M. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulate aortic valve calcification. *J Mol Cell Cardiol.* 2004;36:57-66.
- [54] Steinmetz M, Skowasch D, Wernert N, Welsch U, Preusse CJ, Welz A, Nickenig G, Bauriedel G. Differential profile of the OPG/RANKL/RANK-system in degenerative aortic native and bioprosthetic valves. *J Heart Valve Dis.* 2008;17:187-93.
- [55] Morishima A, Marui A, Shimamoto T, Saji Y, Tambara K, Nishina T, Komeda M. Successful aortic valve replacement for Heyde syndrome with confirmed hematologic recovery. *Ann Thorac Surg.* 2007;83:287-8.
- [56] Pareti FI, Lattuada A, Bressi C, Zanobini M, Sala A, Steffan A, Ruggeri ZM. Proteolysis of von Willebrand factor and shear stress-induced platelet aggregation in patients with aortic valve stenosis. *Circulation.* 2000;102:1290-5.
- [57] Vincentelli A, Susen S, Le Tourneau T, Six I, Fabre O, Juthier F, Bauters A, Decoene C, Goudemand J, Prat A, Jude B. Acquired von Willebrand syndrome in aortic stenosis. *N Engl J Med.* 2003;349:343-9.
- [58] Yoshida K, Tobe S, Kawata M. Acquired von Willebrand disease type IIA in patients with aortic valve stenosis. *Ann Thorac Surg.* 2006;81:1114-6.
- [59] Yoshida K, Tobe S, Kawata M, Yamaguchi M. Acquired and reversible von Willebrand disease with high shear stress aortic valve stenosis. *Ann Thorac Surg.* 2006;81:490-4.
- [60] Olsson M, Hultcrantz R, Schulman S, Wallgren E. Acquired platelet dysfunction may be an aetiological factor in Heyde's syndrome--normalization of bleeding time after aortic valve replacement. *J Intern Med.* 2002;252:516-23.
- [61] Goldsmith IR, Blann AD, Patel RL, Lip GY. Effect of aortic valve replacement on plasma soluble P-selectin, von Willebrand factor, and fibrinogen. *Am J Cardiol.* 2001;87:107-10, A9.
- [62] Moriyama Y, Nakamura K, Kariyazono H, Toyohira H, Taira A. Influence of low-intensity anticoagulation and low-dose antiplatelet agent on coagulation-fibrinolysis system after mechanical prosthetic valve replacement. *J Thorac Cardiovasc Surg.* 1998;115:952-4.
- [63] Iida M, Yamamoto M, Yamazaki M, Sawaguchi M, Honjo H, Kodama I, Kamiya K. Association of aortic valve sclerosis with thrombin generation in hypertensive patients. *J Hum Hypertens.* 2008.
- [64] Marechaux S, Corseaux D, Vincentelli A, Richardson M, Ung A, Susen S, Zawadzki C, Beregi JP, Ezekowitz MD, Jude B, Le Tourneau T. Identification of tissue factor in experimental aortic valve sclerosis. *Cardiovasc Pathol.* 2008.

- [65] Risau W. Angiogenesis and endothelial cell function. *Arzneimittelforschung*. 1994;44:416-7.
- [66] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*. 1996;86:353-64.
- [67] Chen F, Eriksson P, Kimura T, Herzfeld I, Valen G. Apoptosis and angiogenesis are induced in the unstable coronary atherosclerotic plaque. *Coron Artery Dis*. 2005;16:191-7.
- [68] Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. *Br J Surg*. 2001;88:945-50.
- [69] Soini Y, Salo T, Satta J. Angiogenesis is involved in the pathogenesis of nonrheumatic aortic valve stenosis. *Hum Pathol*. 2003;34:756-63.
- [70] Charest A, Pepin A, Shetty R, Cote C, Voisine P, Dagenais F, Pibarot P, Mathieu P. Distribution of SPARC during neovascularisation of degenerative aortic stenosis. *Heart*. 2006;92:1844-9.
- [71] Sage EH. Regulation of interactions between cells and extracellular matrix: a command performance on several stages. *J Clin Invest*. 2001;107:781-3.
- [72] Chalajour F, Treede H, Gehling UM, Ebrahimnejad A, Boehm DH, Riemer RK, Ergun S, Reichenspurner H. Identification and characterization of cells with high angiogenic potential and transitional phenotype in calcific aortic valve. *Exp Cell Res*. 2007;313:2326-35.
- [73] Nagase H, Woessner JF, Jr. Matrix metalloproteinases. *J Biol Chem*. 1999;274:21491-4.
- [74] Edep ME, Shirani J, Wolf P, Brown DL. Matrix metalloproteinase expression in nonrheumatic aortic stenosis. *Cardiovasc Pathol*. 2000;9:281-6.
- [75] Fondard O, Detaint D, Iung B, Choqueux C, Adle-Biasette H, Jarraya M, Hvass U, Couetil JP, Henin D, Michel JB, Vahanian A, Jacob MP. Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors. *Eur Heart J*. 2005;26:1333-41.
- [76] Kaden JJ, Vocke DC, Fischer CS, Grobholz R, Brueckmann M, Vahl CF, Hagl S, Haase KK, Dempfle CE, Borggrefe M. Expression and activity of matrix metalloproteinase-2 in calcific aortic stenosis. *Z Kardiol*. 2004;93:124-30.
- [77] Satta J, Oiva J, Salo T, Eriksen H, Ohtonen P, Biancari F, Juvonen TS, Soini Y. Evidence for an altered balance between matrix metalloproteinase-9 and its inhibitors in calcific aortic stenosis. *Ann Thorac Surg*. 2003;76:681-8; discussion 8.
- [78] Soini Y, Satta J, Maatta M, Autio-Harmainen H. Expression of MMP2, MMP9, MT1-MMP, TIMP1, and TIMP2 mRNA in valvular lesions of the heart. *J Pathol*. 2001;194:225-31.
- [79] Jian B, Jones PL, Li Q, Mohler ER, 3rd, Schoen FJ, Levy RJ. Matrix metalloproteinase-2 is associated with tenascin-C in calcific aortic stenosis. *Am J Pathol*. 2001;159:321-7.
- [80] Yeghiazaryan K, Skowasch D, Bauriedel G, Schild H, Golubnitschaja O. Could activated tissue remodeling be considered as early marker for progressive valve degeneration? Comparative analysis of checkpoint and ECM remodeling gene expression in native degenerating aortic valves and after bioprosthetic replacement. *Amino Acids*. 2007;32:109-14.
- [81] Helske S, Syvaranta S, Kupari M, Lappalainen J, Laine M, Lommi J, Turto H, Mayranpaa M, Werkkala K, Kovanen PT, Lindstedt KA. Possible role for mast cell-derived cathepsin G in the adverse remodelling of stenotic aortic valves. *Eur Heart J*. 2006;27:1495-504.
- [82] Helske S, Syvaranta S, Lindstedt KA, Lappalainen J, Oorni K, Mayranpaa MI, Lommi J, Turto H, Werkkala K, Kupari M, Kovanen PT. Increased expression of elastolytic

- cathepsins S, K, and V and their inhibitor cystatin C in stenotic aortic valves. *Arterioscler Thromb Vasc Biol.* 2006;26:1791-8.
- [83] Kaden JJ. Towards medical therapy of calcific aortic stenosis lessons from molecular biology. *Eur Heart J.* 2007;28:1795-6.
- [84] Mayranpaa MI, Heikkila HM, Lindstedt KA, Walls AF, Kovanen PT. Desquamation of human coronary artery endothelium by human mast cell proteases: implications for plaque erosion. *Coron Artery Dis.* 2006;17:611-21.
- [85] Miller JD, Chu Y, Brooks RM, Richenbacher WE, Pena-Silva R, Heistad DD. Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. *J Am Coll Cardiol.* 2008;52:843-50.
- [86] Liberman M, Bassi E, Martinatti MK, Lario FC, Wosniak J, Jr., Pomerantzeff PM, Laurindo FR. Oxidant generation predominates around calcifying foci and enhances progression of aortic valve calcification. *Arterioscler Thromb Vasc Biol.* 2008;28:463-70.
- [87] Haurani MJ, Pagano PJ. Adventitial fibroblast reactive oxygen species as autocrine and paracrine mediators of remodeling: bellwether for vascular disease? *Cardiovasc Res.* 2007;75:679-89.
- [88] Watson C, Alp NJ. Role of *Chlamydia pneumoniae* in atherosclerosis. *Clin Sci (Lond).* 2008;114:509-31.
- [89] Ieven MM, Hoymans VY. Involvement of *Chlamydia pneumoniae* in atherosclerosis: more evidence for lack of evidence. *J Clin Microbiol.* 2005;43:19-24.
- [90] Porqueddu M, Spirito R, Parolari A, Zanobini M, Pompilio G, Polvani G, Alamanni F, Stangalini D, Tremoli E, Biglioli P. Lack of association between serum immunoreactivity and *Chlamydia pneumoniae* detection in the human aortic wall. *Circulation.* 2002;106:2647-8.
- [91] Agmon Y, Khandheria BK, Jamil Tajik A, Seward JB, Sicks JD, Fought AJ, O'Fallon WM, Smith TF, Wiebers DO, Meissner I. Inflammation, infection, and aortic valve sclerosis; Insights from the Olmsted County (Minnesota) population. *Atherosclerosis.* 2004;174:337-42.
- [92] Atar S, Tolstrup K, Cercek B, Siegel RJ. *Chlamydia pneumoniae* antibody titers and cardiac calcifications: a cross-sectional serological-echocardiographic correlative study. *Isr Med Assoc J.* 2007;9:517-20.
- [93] Kaden JJ, Bickelhaupt S, Grobholz R, Brueckmann M, Haase KK, Dempfle CE, Borggreffe M. Pathogenetic role of *Chlamydia pneumoniae* in calcific aortic stenosis: immunohistochemistry study and review of the literature. *J Heart Valve Dis.* 2003;12:447-53.
- [94] Radke PW, Ortlepp JR, Merkelbach-Bruse S, Kaiser A, Kronenberger S, Handt S, Hanrath P. Prevalence of *Chlamydia pneumoniae* in nonrheumatic stenotic aortic valves. *Am Heart J.* 2002;144:E12.
- [95] Juvonen J, Juvonen T, Laurila A, Kuusisto J, Alarakkola E, Sarkioja T, Bodian CA, Kairaluoma MI, Saikku P. Can degenerative aortic valve stenosis be related to persistent *Chlamydia pneumoniae* infection? *Ann Intern Med.* 1998;128:741-4.
- [96] Rose AG. Failure to detect *Chlamydia pneumoniae* in senile calcific aortic stenosis or calcified congenital bicuspid aortic valve by immunofluorescence, polymerase chain reaction and electron microscopy. *Cardiovasc Pathol.* 2002;11:300-4.
- [97] Bratos-Perez MA, Sanchez PL, Garcia de Cruz S, Villacorta E, Palacios IF, Fernandez-Fernandez JM, Di Stefano S, Orduna-Domingo A, Carrascal Y, Mota P, Martin-Luengo C, Bermejo J, San Roman JA, Rodriguez-Torres A, Fernandez-Aviles F. Association between self-replicating calcifying nanoparticles and aortic stenosis: a possible link to valve calcification. *Eur Heart J.* 2008;29:371-6.
- [98] Maher ER, Young G, Smyth-Walsh B, Pugh S, Curtis JR. Aortic and mitral valve calcification in patients with end-stage renal disease. *Lancet.* 1987;2:875-7.
- [99] McFalls EO, Archer SL. Rapid progression of aortic stenosis and secondary hyperparathyroidism. *Am Heart J.* 1990;120:206-8.

- [100] Lindroos M, Kupari M, Valvanne J, Strandberg T, Heikkilä J, Tilvis R. Factors associated with calcific aortic valve degeneration in the elderly. *Eur Heart J*. 1994;15:865-70.
- [101] Ortlepp JR, Pillich M, Schmitz F, Mevissen V, Koos R, Weiss S, Stork L, Dronsowski R, Langebartels G, Autschbach R, Brandenburg V, Woodruff S, Kaden JJ, Hoffmann R. Lower serum calcium levels are associated with greater calcium hydroxyapatite deposition in native aortic valves of male patients with severe calcific aortic stenosis. *J Heart Valve Dis*. 2006;15:502-8.
- [102] Linhartova K, Veselka J, Sterbakova G, Racek J, Topolcan O, Cerbak R. Parathyroid hormone and vitamin D levels are independently associated with calcific aortic stenosis. *Circ J*. 2008;72:245-50.
- [103] Mohler ER, 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation*. 2001;103:1522-8.
- [104] Rajamannan NM, Subramaniam M, Rickard D, Stock SR, Donovan J, Springett M, Orszulak T, Fullerton DA, Tajik AJ, Bonow RO, Spelsberg T. Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation*. 2003;107:2181-4.
- [105] Pohjolainen V, Taskinen P, Soini Y, Rysa J, Ilves M, Juvonen T, Ruskoaho H, Leskinen H, Satta J. Noncollagenous bone matrix proteins as a part of calcific aortic valve disease regulation. *Hum Pathol*. 2008.
- [106] Rajamannan NM, Nealis TB, Subramaniam M, Pandya S, Stock SR, Ignatiev CI, Sebo TJ, Rosengart TK, Edwards WD, McCarthy PM, Bonow RO, Spelsberg TC. Calcified rheumatic valve neoangiogenesis is associated with vascular endothelial growth factor expression and osteoblast-like bone formation. *Circulation*. 2005;111:3296-301.
- [107] Babu AN, Meng X, Zou N, Yang X, Wang M, Song Y, Cleveland JC, Weyant M, Banerjee A, Fullerton DA. Lipopolysaccharide stimulation of human aortic valve interstitial cells activates inflammation and osteogenesis. *Ann Thorac Surg*. 2008;86:71-6.
- [108] Mallat Z, Tedgui A. Unbalanced RANKL/RANK pathway in aortic valve sclerosis. *J Mol Cell Cardiol*. 2004;36:17-9.
- [109] Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. *JAMA*. 2004;292:490-5.
- [110] Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. *Nat Rev Genet*. 2003;4:638-49.
- [111] Osman L, Chester AH, Sarathchandra P, Latif N, Meng W, Taylor PM, Yacoub MH. A novel role of the sympatho-adrenergic system in regulating valve calcification. *Circulation*. 2007;116:I282-7.
- [112] Osman L, Chester AH, Amrani M, Yacoub MH, Smolenski RT. A novel role of extracellular nucleotides in valve calcification: a potential target for atorvastatin. *Circulation*. 2006;114:I566-72.
- [113] Verrier ED, Boyle EM, Jr. Endothelial cell injury in cardiovascular surgery. *Ann Thorac Surg*. 1996;62:915-22.
- [114] Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol*. 2007;49:2379-93.
- [115] Ridger V, Krams R, Carpi A, Evans PC. Hemodynamic parameters regulating vascular inflammation and atherosclerosis: a brief update. *Biomed Pharmacother*. 2008;62:536-40.
- [116] Helderma F, Segers D, de Crom R, Hierck BP, Poelmann RE, Evans PC, Krams R. Effect of shear stress on vascular inflammation and plaque development. *Curr Opin Lipidol*. 2007;18:527-33.

- [117] Harrison DG, Widder J, Grumbach I, Chen W, Weber M, Searles C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med*. 2006;259:351-63.
- [118] Partridge J, Carlsen H, Enesa K, Chaudhury H, Zakkar M, Luong L, Kinderlerer A, Johns M, Blomhoff R, Mason JC, Haskard DO, Evans PC. Laminar shear stress acts as a switch to regulate divergent functions of NF-kappaB in endothelial cells. *FASEB J*. 2007;21:3553-61.
- [119] Hastings NE, Simmers MB, McDonald OG, Wamhoff BR, Blackman BR. Atherosclerosis-prone hemodynamics differentially regulates endothelial and smooth muscle cell phenotypes and promotes pro-inflammatory priming. *Am J Physiol Cell Physiol*. 2007;293:C1824-33.
- [120] Gelosa P, Cimino M, Pignieri A, Tremoli E, Guerrini U, Sironi L. The role of HMG-CoA reductase inhibition in endothelial dysfunction and inflammation. *Vasc Health Risk Manag*. 2007;3:567-77.
- [121] Werba JP, Tremoli E, Massironi P, Camera M, Cannata A, Alamanni F, Biglioli P, Parolari A. Statins in coronary bypass surgery: rationale and clinical use. *Ann Thorac Surg*. 2003;76:2132-40.
- [122] Liebe V, Brueckmann M, Borggrefe M, Kaden JJ. Statin therapy of calcific aortic stenosis: hype or hope? *Eur Heart J*. 2006;27:773-8.
- [123] Rajamannan NM, Subramaniam M, Caira F, Stock SR, Spelsberg TC. Atorvastatin inhibits hypercholesterolemia-induced calcification in the aortic valves via the Lrp5 receptor pathway. *Circulation*. 2005;112:I229-34.
- [124] Rajamannan NM, Subramaniam M, Stock SR, Stone NJ, Springett M, Ignatiev KI, McConnell JP, Singh RJ, Bonow RO, Spelsberg TC. Atorvastatin inhibits calcification and enhances nitric oxide synthase production in the hypercholesterolaemic aortic valve. *Heart*. 2005;91:806-10.
- [125] Wu B, Elmariah S, Kaplan FS, Cheng G, Mohler ER, 3rd. Paradoxical effects of statins on aortic valve myofibroblasts and osteoblasts: implications for end-stage valvular heart disease. *Arterioscler Thromb Vasc Biol*. 2005;25:592-7.
- [126] Anger T, El-Chafchak J, Habib A, Stumpf C, Weyand M, Daniel WG, Hombach V, Hoeher M, Garlich CD. Statins stimulate RGS-regulated ERK 1/2 activation in human calcified and stenotic aortic valves. *Exp Mol Pathol*. 2008.
- [127] Cowell SJ, Newby DE, Prescott RJ, Bloomfield P, Reid J, Northridge DB, Boon NA. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med*. 2005;352:2389-97.
- [128] Moura LM, Ramos SF, Zamorano JL, Barros IM, Azevedo LF, Rocha-Goncalves F, Rajamannan NM. Rosuvastatin affecting aortic valve endothelium to slow the progression of aortic stenosis. *J Am Coll Cardiol*. 2007;49:554-61.
- [129] Rossebø AB, Pedersen TR, Boman K, Brudi P, Chambers JB, Egstrup K, Gerdts E, Gohlke-Barwolf C, Holme I, Kesaniemi YA, Malbecq W, Nienaber CA, Ray S, Skjaerpe T, Wachtell K, Willenheimer R. Intensive Lipid Lowering with Simvastatin and Ezetimibe in Aortic Stenosis. *N Engl J Med*. 2008.
- [130] Peto R, Emberson J, Landray M, Baigent C, Collins R, Clare R, Califf R. Analyses of Cancer Data from Three Ezetimibe Trials. *N Engl J Med*. 2008.
- [131] Chan KL, Teo K, Tam J, Dumesnil JG. Rationale, design, and baseline characteristics of a randomized trial to assess the effect of cholesterol lowering on the progression of aortic stenosis: the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) trial. *Am Heart J*. 2007;153:925-31.

- [132] Novaro GM, Tiong IY, Pearce GL, Lauer MS, Sprecher DL, Griffin BP. Effect of hydroxymethylglutaryl coenzyme a reductase inhibitors on the progression of calcific aortic stenosis. *Circulation*. 2001;104:2205-9.
- [133] Aronow WS, Ahn C, Kronzon I, Goldman ME. Association of coronary risk factors and use of statins with progression of mild valvular aortic stenosis in older persons. *Am J Cardiol*. 2001;88:693-5.
- [134] Bellamy MF, Pellicka PA, Klarich KW, Tajik AJ, Enriquez-Sarano M. Association of cholesterol levels, hydroxymethylglutaryl coenzyme-A reductase inhibitor treatment, and progression of aortic stenosis in the community. *J Am Coll Cardiol*. 2002;40:1723-30.
- [135] Rosenhek R, Rader F, Loho N, Gabriel H, Heger M, Klaar U, Schemper M, Binder T, Maurer G, Baumgartner H. Statins but not angiotensin-converting enzyme inhibitors delay progression of aortic stenosis. *Circulation*. 2004;110:1291-5.
- [136] Shavelle DM, Takasu J, Budoff MJ, Mao S, Zhao XQ, O'Brien KD. HMG CoA reductase inhibitor (statin) and aortic valve calcium. *Lancet*. 2002;359:1125-6.
- [137] O'Brien KD, Zhao XQ, Shavelle DM, Caulfield MT, Letterer RA, Kapadia SR, Probstfield JL, Otto CM. Hemodynamic effects of the angiotensin-converting enzyme inhibitor, ramipril, in patients with mild to moderate aortic stenosis and preserved left ventricular function. *J Investig Med*. 2004;52:185-91.
- [138] Antonini-Canterin F, Hirsu M, Popescu BA, Leiballi E, Piazza R, Pavan D, Gingham C, Nicolosi GL. Stage-related effect of statin treatment on the progression of aortic valve sclerosis and stenosis. *Am J Cardiol*. 2008;102:738-42.
- [139] Antonini-Canterin F, Corrado G, Faggiano P, Popescu BA, Carerj S, La Carrubba S, Zuppiroli A, Nicolosi GL. A medical therapy for aortic valve sclerosis and aortic valve stenosis? Rationale of the ASSIST study (Asymptomatic aortic Sclerosis/Stenosis: Influence of STatins): a large, observational, prospective, multicenter study of the Italian Society of Cardiovascular Echography. *J Cardiovasc Med (Hagerstown)*. 2006;7:464-9.
- [140] Helske S, Lindstedt KA, Laine M, Mayranpaa M, Werkkala K, Lommi J, Turto H, Kupari M, Kovanen PT. Induction of local angiotensin II-producing systems in stenotic aortic valves. *J Am Coll Cardiol*. 2004;44:1859-66.
- [141] Arishiro K, Hoshiga M, Negoro N, Jin D, Takai S, Miyazaki M, Ishihara T, Hanafusa T. Angiotensin receptor-1 blocker inhibits atherosclerotic changes and endothelial disruption of the aortic valve in hypercholesterolemic rabbits. *J Am Coll Cardiol*. 2007;49:1482-9.

## **2.4 Do statins improve outcomes and delay the progression of non-rheumatic calcific aortic stenosis?**

### **INTRODUCTION**

Non-rheumatic calcific aortic stenosis is the commonest valve disease in adults and the third most common cardiovascular diagnosis (after hypertension and need for coronary bypass surgery).[1] Surgery remains the only treatment option in advanced stages.[1] As several studies have indicated that aortic stenosis progression is due to an active process sharing several features with atherosclerosis, several trials have assessed the role of statins in delaying such progression, but with conflicting results.[2, 3] Since 2001, several studies, mainly observational, suggested that statin therapy delays the progression of calcific non-rheumatic aortic stenosis, assessed by echocardiography or computed tomography. These findings were not confirmed by three recent prospective randomized trials.[4, 5, 6] For this reason, the role of statins in these patients is an open question. We have undertaken a meta-analysis of studies of the effect of statins on the incidence of hard end-points and on retarding stenosis progression.

### **METHODS**

The meta-analysis used PRISMA,[7] and MOOSE guidelines.[8] We performed a computerized literature search of Medline and PubMed up to January 2010, supplemented with manual bibliography reviews. The following free text search string (formatted for PubMed) was used: [(statins OR Hydroxymethylglutaryl coenzyme-a reductase inhibitors) AND aortic]. We used tangential electronic exploration of related articles and manual searches of bibliographies, related journals, and reference lists of reviews. All peer-reviewed studies published reporting the effect of statin therapy on outcomes and on valve stenosis progression in patients with calcific non-rheumatic aortic stenosis were identified. The ability



of the search strategy to identify four relevant studies was tested and found fit for purpose. All titles and abstracts of the identified articles were examined by two investigators (AP, DP) for potential eligibility for subsequent analysis.

The first step of the analysis was to collect all the trials conforming to the following criteria:

1. Studies comparing the mid- or long-term effects ( $\geq 1$  year) of statin therapy vs. placebo or no statins in patients affected by calcific non-rheumatic aortic stenosis;
2. Data concerning mid- or long-term hard outcomes or about the progression of aortic stenosis had to be reported in the study.

The following hard endpoints were collected:

1. Death from any cause at follow-up (n/y);
2. Death from cardiovascular causes at follow-up (n/y);
3. Need to undergo aortic valve surgery at follow-up (n/y).

Concerning aortic valve stenosis progression, the following variables were searched:

1. Peak aortic-jet velocity progression (m/s/y);
2. Aortic valve area decrease (cm<sup>2</sup>/y);
3. Peak aortic gradient progression (mmHg/y);
4. Mean aortic gradient progression (mmHg/y);

The outcome definitions used by the original researchers were accepted. Bibliographies of included articles were also searched.

Several strategies were employed to avoid duplication of data. If the same institution had produced several studies, only those reporting recruitment times were considered. If there was sample overlap between studies, only the largest study was included. Data were abstracted and analyzed by two authors (AP, MT), and disagreements were resolved by consensus.

**Analyses.** Data were analyzed by means of RevMan 5 (RevMan 5.0.22, Cochrane Collaboration, Oxford, United Kingdom) and Comprehensive Meta-analysis Version 2

(Biostat, Englewood, NJ). Effects on dichotomous outcomes were expressed as odds ratio (OR) with 95% confidence intervals (CI). Effects on continuous variables were expressed as mean difference with 95% CI.

Heterogeneity was assessed with the  $\text{Chi}^2$  test; in addition, the  $I^2$  was calculated to quantify the degree of heterogeneity across trials that could not be attributable to chance alone.  $I^2$ , the *proportion* of variability not attributable to chance alone, provides an improved measure of heterogeneity between trials and is not limited by power.[9] When there was no significant heterogeneity, treatment effects were pooled with the fixed-effects model, but if there was significant ( $p \leq 0.1$ ) heterogeneity in the main analysis or in a subanalysis, the random-effects model was used; when a random-effects analysis was performed, among-study variance was also assessed with  $\text{Tau}^2$  statistic.

Subanalyses, defined *a priori*, were the analyses of prospective and retrospective studies, and of randomized and nonrandomized studies done separately. In addition, the effect of statin treatment on aortic stenosis progression parameters was assessed in studies that enrolled patients with average LDL cholesterol levels  $\leq 130$  mg/dL. Finally, weighted fixed-effects meta-regression was used to examine the possibility of effect modification on aortic stenosis progression by duration of statin treatment.

Sensitivity of the meta-analyses was assessed after removal of studies in which the largest (or smallest) effect was found and of the study with the largest number of patients. In addition, we performed random-effects meta-analysis on the outcomes of interest.

Publication bias was explored through visual inspection of funnel plots, and by one-tailed Egger's test. Other than for the Q statistic, statistical significance was defined by  $p \leq 0.05$ .

## RESULTS

### **Selected studies**

The selection criteria described under Methods were applied to 812 studies identified by the literature searches, the publications were examined, and 21 candidate trials were identified for further assessment (see online supplementary data for bibliography). Of these, 6 were discarded as they were not focused on therapy with statins. On further examination of these 15 remaining studies, 5 were excluded either because they did not report any extractable data, because of possible duplicate publication, or because they were focused on rheumatic aortic stenosis. Of the 10 studies finally selected for meta-analysis (Table 1), 5 were prospective, 5 retrospective, whereas 3 were randomized, and 7 not randomized, respectively. One of the retrospective, non-randomized studies[10] reported separately the outcomes for patients affected by mild aortic stenosis and moderate aortic stenosis, and for that reason data concerning these subcategories of patients were inserted separately into the meta-analysis. In the end, a total of 3822 participants (2214 non statin-treated and 1608 statin-treated) in 10 studies provided data for this meta-analysis.

**Table 1**  
**Characteristics of included trials**

Study	Year	Study design	Treatment groups	No. of pts.	Age (y)	LDL cholesterol (mg/dL)	Diabetes	Aortic jet velocity (m/sec)	Aortic valve area (cm <sup>2</sup> )	Transaortic pressure gradient (mmHg)	Follow-up
Chan (ASTRONOMER) [6]	2010	Prospective randomized	Placebo	135	58±14	122±29	n.a.	3.19±0.42	1.56±0.70	Mean:23±8	Median 3.5 (2.1-4.5) years
			Rosuvastatin 40 mg/d	134	58±13	124±25	n.a.	3.16±0.42	1.49±0.71	Mean:23±8	
Cowell (SALTIRE) [4]	2005	Prospective randomized	Placebo	78	68±10	133±30	4 (5%)	3.45±0.67	1.02±0.41	Peak:50±20	Median: 25 (7-36) months
			Atorvastatin 80 mg/d	77	68±10	137±34	3 (4%)	3.39±0.62	1.03±0.40	Peak:48±17	
Mohler [11]	2007	Prospective observational	No statins	22	64±10	} 110±33	3 (14%)	n.a.	1.22±0.25	n.a.	12 months
			Statins	31	70±10		7 (18%)	n.a.	1.13±0.27	n.a.	
Moura (RAAVE) [12]	2007	Prospective open label	No treatment	60	74±9	117±21	13 (22%)	3.62±0.61	1.20±0.35	Mean:36±13	Mean: 73±24 weeks
			Rosuvastatin 20 mg/d	61	73±9	158±32	26 (43%)	3.65±0.64	1.23±0.43	Mean:35±13	
Rossebo (SEAS) [5]	2008	Prospective randomized	Placebo	929	67±10	139±35	n.a.	3.10±0.54	1.27±0.46	Mean:23.0±8.7	Median: 52.2 months
			Simvastatin (40-80 mg/d) plus ezetimibe	944	68±9	140±36	n.a.	3.09±0.55	1.29±0.48	Mean:22.7±8.8	
Antonini-Canterin [10]	2008	Retrospective	No statins: mild aortic stenosis	360	71±8	n.a.	82 (23%)	2.3±0.2	n.a.	Mean:13.1±3.2	Mean: 5.6±3.2 years
			Statins: mild aortic stenosis	141	71±7	n.a.	50 (36%)	2.3±0.2	n.a.	Mean:12.7±3.1	
			No statins: moderate aortic stenosis	214	72±8	n.a.	42 (20%)	3.3±0.2	n.a.	Mean:26.1±5.1	
			Statins: moderate aortic stenosis	62	70±8	n.a.	14 (23%)	3.3±0.2	n.a.	Mean:26.0±5.0	
Bellamy [13]	2002	Retrospective	No statins	118	78±12	137±43	28 (24%)	3.0±0.8	1.20±0.35	Mean:22±12	Mean: 3.7±2.3 years
			Statins	38	73±11	164±49	9 (24%)	2.8±0.5	1.32±0.29	Mean:18±7	
Kuwabara [14]	2006	Retrospective	No statins	20	75±5	n.a.	3 (15%)	} 3.1±1.0	n.a	} Mean:42±29	31±23 months
			Statins	13	74±5	n.a.	6 (46%)		n.a.		30±20 months

Novaro [15]	2001	Retrospective	No statins Statins	117 57	67±13 71±9	131(112-143) 128(94-146)	23 (20%) 20 (35%)	n.a. n.a.	1.2(1.0-1.4) 1.2(1.0-1.4)	Mean:15(12-22) Mean:15(12-22)	Mean:21 months
Rosenhek [16]	2004	Retrospective	No statins Statins	161 50	69±11 72±8	141±39 145±38	32 (20%) 11 (22%)	3.92±0.86 4.08±0.86	0.84±0.23 0.82±0.23	Mean:42±20 Mean:42±18	Median: 24±18 months

Footnote to Table 1: pts.= patients; n.a.= not available

## Meta-analysis

As to outcomes, there were no differences between statin-treated and untreated patients. All-cause mortality (OR 0.98; 95% CI 0.74, 1.30, figure 2 panel A), cardiovascular mortality (OR 0.79; 95% CI 0.54, 1.15, figure 2 panel B), and the need for aortic valve surgery (OR 0.92; 95% CI 0.76, 1.10, figure 2 panel C) were not statistically significantly reduced. Notably, data concerning hard outcomes were available only in prospective but not in retrospective trials.

Concerning the progression of aortic valve stenosis over time (Tables 2 and 3), the analysis of the variables (jet velocity progression, mean annual decrease in aortic valve area, peak and mean aortic gradient progression) that were considered gave consistent results showing that a possible protective effect of statins is supported only by low quality studies (retrospective or non-randomized), but not by high quality-ones (prospective and randomized).

In fact, although the overall effect of statin treatment on the mean annual difference of jet velocity progression ( $-0.08$  m/s/y, 95% CI  $-0.13$ ,  $-0.03$ ,  $p=0.0007$ , figure 3 panel A) and the mean annual difference in aortic valve area, ( $-0.02$  cm<sup>2</sup>/y, 95% CI  $-0.03$ ,  $0.00$ ,  $p=0.02$ , figure 3 panel B) was statistically significant, the subanalyses based on the quality of studies showed that the effect was driven only by lower quality studies whereas in higher quality ones there was no effect. The progression of peak aortic gradient ( $-1.76$  mmHg/y, 95%CI  $-3.73$ ,  $0.21$ ;  $p=0.08$ , figure 4 panel A) and mean aortic gradient ( $-0.99$  mmHg/y, 95%CI  $-2.04$ ,  $0.07$ ,  $p=0.07$  figure 4 panel B) was slightly lower in statin-treated patients, but this did not reach statistical significance; also in this case the discrepancy in statin effect between high- and low-quality studies was evident, being only lower quality studies in favor of statin treatment. Finally, there was heterogeneity in the analyses concerning the progression of jet velocity, of peak and mean aortic gradient; and the inspection of funnel plots in these cases showed asymmetry suggesting publication bias (the funnel plots are available from the author upon request) with a significant Egger's test (Tables 2 and 3).

Concerning the analysis of statins effect on aortic stenosis progression parameters in studies that enrolled patients with average LDL cholesterol levels  $\leq 130$  mg/dL, only three out of ten studies were available for final analysis, and data were available for aortic valve area decrease over time, and for peak and mean aortic gradient progression, but not for jet velocity progression. Statin treatment did not affect these parameters (please see supplementary data eFigure2, panels A-C), but the limits due to a small sample size should be considered. Finally, meta-regression analyses did not support a role of statin treatment duration in aortic stenosis progression.

**Table 2**  
**Clinical outcomes by prospective and retrospective studies**

Hard outcomes	n (N)	Events		OR (95% CI)	p for overall effect	Heterogeneity		Egger's test p value
		Statins	No statins			p value (Q statistic)	I <sup>2</sup>	
Death from any cause (only prospective studies available)	2149 (3)	109/1082 (10.1%)	109/1067 (10.2%)	0.98 (0.74, 1.30)	0.91	0.33	9%	0.15
Death from cardiovascular causes (only prospective studies available)	2297 (3)	52/1155 (4.5%)	64/1142 (5.6 %)	0.79 (0.54, 1.15)	0.22	0.67	0%	0.70
Aortic valve surgery (only prospective studies available)	2418 (4)	311/1216 (25.6%)	327/1202 (27.2%)	0.92 (0.76, 1.10)	0.35	0.43	0%	0.99

Aortic valve stenosis progression	n (N)	Mean difference (95% CI)	p for overall effect	Heterogeneity		Egger's test p value
				p value (Q statistic)	I <sup>2</sup>	
Jet velocity progression (m/s/y)	3125 (7)	-0.08 (-0.13, -0.03)	0.0007	<0.00001	82%	0.02
Prospective studies	1948 (3)	-0.05 (-0.13, 0.03)	0.22	0.01	78%	
Retrospective studies	1177 (4)	-0.11 (-0.17, -0.04)	0.002	0.0002	82%	
Aortic valve area decrease (sq cm/y)	2608 (7)	-0.02 (-0.03, 0.00)	0.02	0.24	25%	0.21
Prospective studies	2278 (5)	-0.01 (-0.03, 0.00)	0.15	0.29	19%	
Retrospective studies	330 (2)	-0.05 (-0.09, -0.01)	0.01	1.00	0%	
Peak aortic gradient progression (mm Hg/y)	731 (5)	-1.76 (-3.73, 0.21)	0.08	0.05	57%	0.03
Prospective studies	524 (3)	-0.67 (-2.89, 1.54)	0.55	0.15	47%	
Retrospective studies	207 (2)	-3.08 (-5.22, -0.94)	0.005	0.34	0%	
Mean aortic gradient progression (mm Hg/y)	2413 (5)	-0.99 (-2.04, 0.07)	0.07	0.008	71%	0.04
Prospective studies	2083 (3)	-0.36 (-1.25, 0.53)	0.43	0.12	53%	
Retrospective studies	330 (2)	-1.92 (-3.55, -0.29)	0.02	0.22	35%	

Footnote to Table 2: n = number of patients; N = number of trials; OR = odds ratio; CI = confidence interval



**Table 3**  
**Clinical outcomes by randomized and nonrandomized studies**

Hard outcomes	n (N)	Events		OR (95% CI)	p for overall effect	Heterogeneity		Egger's test p value
		Statins	No statins			p value (Q statistic)	I <sup>2</sup>	
Death from any cause	2149 (3)	109/1082 (10.1%)	109/1067 (10.2%)	0.98 (0.74, 1.30)	0.91	0.33	9%	0.15
Randomized studies	2028 (2)	108/1021 (10.6%)	105/1007 (10.4%)	1.01 (0.76, 1.35)	0.92	0.46	0%	
Non-randomized studies	121 (1)	1/61 (1.6%)	4/60 (6.7%)	0.23 (0.03, 2.15)	0.20	n.a.	n.a.	
Death from cardiovascular causes (only randomized studies available)	2297 (3)	52/1155 (4.5%)	64/1142 (5.6 %)	0.79 (0.54, 1.15)	0.22	0.67	0%	0.70
Aortic valve surgery	2418 (4)	311/1216 (25.6%)	327/1202 (27.2%)	0.92 (0.76, 1.10)	0.35	0.43	0%	0.99
Randomized studies	2297 (3)	306/1155 (26.5%)	324/1142 (28.4%)	0.91 (0.76, 1.09)	0.30	0.35	4%	
Non-randomized studies	121 (1)	5/61 (8.2%)	3/60 (5.0%)	1.70 (0.39, 7.44)	0.48	n.a.	n.a.	

Aortic valve stenosis progression	n (N)	Mean difference (95% CI)	p for overall effect	Heterogeneity		Egger's test p value
				p value (Q statistic)	I <sup>2</sup>	
Jet velocity progression (m/s/y)	3125 (7)	-0.08 (-0.13, -0.03)	0.0007	<0.00001	82%	0.02
Randomized studies	1827 (2)	-0.01 (-0.03, 0.02)	0.48	0.88	0%	
Non-randomized studies	1298(5)	-0.12 (-0.18, -0.06)	0.0002	<0.0001	81%	
Aortic valve area decrease (sq cm/y)	2608 (7)	-0.02 (-0.03, 0.00)	0.02	0.24	25%	0.21
Randomized studies	2096 (3)	0.00 (-0.02, 0.02)	0.75	0.93	0%	
Non-randomized studies	512 (4)	-0.05 (-0.07, -0.02)	0.0004	0.90	0%	
Peak aortic gradient progression (mm Hg/y)	731 (5)	-1.76 (-3.73, 0.21)	0.08	0.05	57%	0.03
Randomized studies	403 (2)	0.10 (-1.36, 1.56)	0.89	0.86	0%	
Non-randomized studies	328 (3)	-3.40 (-5.39, -1.41)	0.0008	0.46	0%	
Mean aortic gradient progression (mm Hg/y)	2413 (5)	-0.99 (-2.04, 0.07)	0.07	0.008	71%	0.04
Randomized studies	1962 (2)	-0.10 (-0.36, 0.16)	0.46	1.00	0%	
Non-randomized studies	451 (3)	-2.19 (-3.35, -1.03)	0.0002	0.38	0%	

Footnote to eTable 1: n = number of patients; N = number of trials; OR = odds ratio; CI = confidence interval

## DISCUSSION

Non-rheumatic calcific aortic stenosis is the leading valve disease of Western countries and is closely related to aging. Less invasive techniques for valve replacement (i.e. transcatheter aortic valve implantation) have recently been introduced in clinical practice,[17] and these techniques will allow further growth in aortic valve surgical procedures in a wider population of older patients. This, however, will cause an increase in health expenditure for this disease that now is estimated to be around 1 billion US dollars per year in the USA.[18] Thus, the possibility of reducing the progression of this disease with drug therapy is consequently of great interest for all health organizations.

The assumption that calcific aortic stenosis is only a passive, age-related disease has been strongly questioned by numerous studies showing that this disease has several biological pathways in common with atherosclerosis, [3] which is why investigators have assessed the effect of statins in this type of patients.

Statins reduce the occurrence of ischemic cardiovascular events not only in secondary prevention but also in high-risk otherwise healthy patients who are candidates for primary prevention.[19] In addition, evidence from the JUPITER trial suggests that statin treatment in patients with normal cholesterol levels but elevated C-reactive protein reduces the risk of cardiovascular disease mortality and morbidity by almost half,[20] showing that, besides their cholesterol-lowering effects, statins possess pronounced anti-inflammatory and anticoagulant effects.[21, 22] Statins might therefore modulate the progression of non-rheumatic calcific aortic stenosis in three ways: by reducing cholesterol levels, a well-known risk factor for aortic stenosis;[3] by attenuating the inflammatory burst within valve tissue;[3] and by modulating systemic,[3] and local [23] hemostatic changes involved in the course of the disease.

Studies investigating the clinical results of statin treatment have given contradictory results. The early enthusiastic findings of observational studies documenting a reduction of the progression of the disorder have been seriously questioned by later, randomized studies, which showed substantial equivalence between statin- and placebo-treated patients.

The meta-analysis reported here shows that, on the basis of data published to date, statin treatment: 1) does not affect the occurrence, at a mid-term follow-up (average 4 years), of major events in patients affected by calcific non-rheumatic aortic stenosis; 2) might reduce the rate of aortic stenosis progression over time, but current evidence is not sufficient at all to warrant statin use in these patients, as it comes only from retrospective, nonrandomized studies, whereas prospective and randomized studies do not show any protective effects of statins on aortic stenosis progression.

Thus, although statins have well-established effects on major end-points in several patient populations, both in primary and secondary prevention, they do not have the same effect on major end points in patients with calcific aortic stenosis. In fact, our meta-analysis of current evidence shows that statins do not reduce total mortality, cardiovascular mortality or the need for valve surgery after 24-48 months of treatment in this cohort of patients. However, the number of patients that could be pooled in this analysis was rather small, and multicenter, prospective, randomized studies are needed to clarify the issue definitively. We estimate that a sample size of 6220 and of 11150 patients for each treatment arm would be needed to demonstrate statistically significant differences between treatments in cardiovascular mortality and need for aortic valve surgery, respectively, at mid-term (mean 39 months) follow-up ( $\alpha$  0.05, power=0.80).

In the patients in our meta-analysis, the effect of statin treatment on hard outcomes was distinctly inferior to that obtained in the JUPITER trial, where the incidence of major outcomes was halved by statin therapy in patients with normal cholesterol but with elevated

inflammatory markers. We hypothesize that statins are most effective during the early stages of stenosis, when inflammation is most active, whereas in the patients we studied the degree of stenosis was far more advanced, as shown by an average jet velocity in all studies  $> 3$  m/sec and by an average aortic valve area between 1 and  $1.4 \text{ cm}^2$  (Table 1). And this hypothesis is also supported by a recent post-hoc analysis of SEAS trial data documenting that in patients affected by calcific aortic stenosis the protection provided by statin treatment is greater in patients with milder aortic stenosis degrees.[24]

Against the hypothesis of inflammation contribution to aortic stenosis progression is the finding that C-reactive protein has been localized in valve tissue of calcific aortic stenosis and that statin-treated patients show lower valve levels of this marker.[25] However, there is some doubt whether there is an association between C-reactive protein and disease progression.[26],[27]

Our meta-analysis also shows that statin treatment might have, if any, a little role in reducing the progression of aortic stenosis over time, with an annual estimated reduction of  $0.08 \text{ m/s/y}$  for peak jet velocity, and an annual reduction of  $0.02 \text{ cm}^2/\text{y}$  for aortic valve area decrease. Otto et al.[28] showed that, in asymptomatic patients affected by calcific aortic stenosis (average valve area of  $1.3 \text{ cm}^2$  and average aortic jet velocity of  $3.6 \text{ m/s}$ ), the approximate annual increase in jet velocity is  $0.3 \text{ m/s}$  with a corresponding decrease of about  $0.1 \text{ cm}^2$  in valve area. Based on these data, statin treatment in the patient population of our meta-analysis might reduce aortic stenosis progression by an estimated 20%-25%. This finding, however, is not confirmed by the behavior of peak and mean aortic gradient; the progression of both these variables was slightly affected by statins, but this was not statistically significant.

In addition, the subanalyses performed on data coming from prospective vs. retrospective studies, and from randomized vs. non-randomized trials, constantly showed that, for all the

four variables representing aortic stenosis progression, the effect of statin treatment was evident only in the studies of lower quality (retrospective and non-randomized), whereas no effect was demonstrated in high-quality ones (prospective or randomized).

We conclude that a beneficial effect of statins on disease progression is supported only by observational studies, with all their inherent flaws, and this is not sufficient to support statin use in patients affected by moderate or by higher grades of nonrheumatic calcific aortic stenosis. Future randomized studies will need to address three deficiencies in those currently available: (1) lack of statistical power in detecting any reduction in cardiovascular mortality; (2) too short follow-up periods, given that the disease process takes many decades and only about 9% of patients in patients aged 65-75 years with aortic sclerosis progress to aortic stenosis in 5 years;[29] and (3) insufficient discrimination in the selection of patients most likely to benefit from preventive statin therapy. It has been previously hypothesized that, in studies conducted up till now, statin therapy was initiated much too late in the course of disease, and that statin therapy should be initiated at an earlier stage of the disease to be effective.[2, 3] Actually, there are insufficient data to support or confute this hypothesis, although this meta-analysis shows that, in patients enrolled at a relatively advanced stage of the disease, statins are not effective in the prevention of cardiovascular events or in the reduction of disease progression. The efficacy of statin therapy needs to be tested in earlier stages of the disease when the transvalvular gradient has not yet ensued, as already suggested by Wierzbicki and coll., [30] and by a study included in our meta-analysis [10] showing that statins effectively delayed the progression of aortic stenosis in patients with aortic sclerosis and mild aortic stenosis, but not in patients with moderate stenosis. In other words, statin therapy as an effective preventive strategy should now be assessed very early in patients at risk of calcific aortic valve disease, and certainly no later than at first diagnosis of aortic

sclerosis or mild stenosis; and periodic screening with transthoracic echocardiography can help in identifying patients at risk.

In addition, it is possible that the discrepancy between the mild or null effects of statin therapy in moderate aortic stenosis, when the disease is already at an advanced stage, and the more evident results that can be achieved by statin treatment in case of earlier stages of atherosclerotic disease (JUPITER trial) can be dissolved by the hypothesis that the maximal efficacy of these drugs might be achieved when they are given at earlier stages of both diseases, aortic stenosis and atherosclerosis.

In conclusion, our study does not support the concept that statins may reduce the progression rate of calcific non-rheumatic aortic stenosis; it also strongly indicates the need for adequately powered, well-designed prospective randomized controlled studies in patients at earlier disease stage.

## REFERENCES

- [1] Carabello BA, Paulus WJ. Aortic stenosis. *Lancet* 2009;**373**:956-66.
- [2] Helske S, Otto CM. Lipid lowering in aortic stenosis: still some light at the end of the tunnel? *Circulation* 2009;**119**:2653-5.
- [3] Parolari A, Loardi C, Mussoni L, et al. Nonrheumatic calcific aortic stenosis: an overview from basic science to pharmacological prevention. *Eur J Cardiothorac Surg* 2009;**35**:493-504.
- [4] Cowell SJ, Newby DE, Prescott RJ, et al. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med* 2005;**352**:2389-97.
- [5] Rossebo AB, Pedersen TR, Boman K, et al. Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *N Engl J Med* 2008;**359**:1343-56.
- [6] Chan KL, Teo K, Dumesnil JG, et al. Effect of Lipid Lowering With Rosuvastatin on Progression of Aortic Stenosis. Results of the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) Trial *Circulation* 2010;**121**:306-14.
- [7] Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med* 2009;**151**:W65-94.
- [8] Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;**283**:2008-12.
- [9] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**:557-60.
- [10] Antonini-Canterin F, Hirsu M, Popescu BA, et al. Stage-related effect of statin treatment on the progression of aortic valve sclerosis and stenosis. *Am J Cardiol* 2008;**102**:738-42.
- [11] Mohler ER, 3rd, Wang H, Medenilla E, et al. Effect of statin treatment on aortic valve and coronary artery calcification. *J Heart Valve Dis* 2007;**16**:378-86.
- [12] Moura LM, Ramos SF, Zamorano JL, et al. Rosuvastatin affecting aortic valve endothelium to slow the progression of aortic stenosis. *J Am Coll Cardiol* 2007;**49**:554-61.
- [13] Bellamy MF, Pellikka PA, Klarich KW, et al. Association of cholesterol levels, hydroxymethylglutaryl coenzyme-A reductase inhibitor treatment, and progression of aortic stenosis in the community. *J Am Coll Cardiol* 2002;**40**:1723-30.
- [14] Kuwabara M, Kitaoka H, Okawa M, et al. Treatment with HMG-CoA reductase inhibitors (statins) attenuates the progression of aortic valve stenosis in the elderly. *Geriatr Gerontol Int* 2006;**6**:124-8.
- [15] Novaro GM, Tiong IY, Pearce GL, et al. Effect of hydroxymethylglutaryl coenzyme a reductase inhibitors on the progression of calcific aortic stenosis. *Circulation* 2001;**104**:2205-9.
- [16] Rosenhek R, Rader F, Loho N, et al. Statins but not angiotensin-converting enzyme inhibitors delay progression of aortic stenosis. *Circulation* 2004;**110**:1291-5.
- [17] Webb JG, Altwegg L, Boone RH, et al. Transcatheter aortic valve implantation: impact on clinical and valve-related outcomes. *Circulation* 2009;**119**:3009-16.
- [18] Moura LM, Maganti K, Puthumana JJ, et al. New understanding about calcific aortic stenosis and opportunities for pharmacologic intervention. *Curr Opin Cardiol* 2007;**22**:572-7.

- [19] Brugts JJ, Yetgin T, Hoeks SE, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ* 2009;**338**:b2376.
- [20] Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008;**359**:2195-207.
- [21] Gelosa P, Cimino M, Pignieri A, et al. The role of HMG-CoA reductase inhibition in endothelial dysfunction and inflammation. *Vasc Health Risk Manag* 2007;**3**:567-77.
- [22] Greenwood J, Mason JC. Statins and the vascular endothelial inflammatory response. *Trends Immunol* 2007;**28**:88-98.
- [23] Marechaux S, Corseaux D, Vincentelli A, et al. Identification of tissue factor in experimental aortic valve sclerosis. *Cardiovasc Pathol* 2009;**18**:67-76.
- [24] Holme I, Boman K, Brudi P, et al. Observed and predicted reduction of ischemic cardiovascular events in the Simvastatin and Ezetimibe in Aortic Stenosis trial. *Am J Cardiol* 2010;**105**:1802-8.
- [25] Skowasch D, Schrempf S, Preusse CJ, et al. Tissue resident C reactive protein in degenerative aortic valves: correlation with serum C reactive protein concentrations and modification by statins. *Heart* 2006;**92**:495-8.
- [26] Jeevanantham V, Singh N, Izuora K, et al. Correlation of high sensitivity C-reactive protein and calcific aortic valve disease. *Mayo Clin Proc* 2007;**82**:171-4.
- [27] Gring CN, Houghtaling P, Novaro GM, et al. Preoperative cholesterol levels do not predict explant for structural valve deterioration in patients undergoing bioprosthetic aortic valve replacement. *J Heart Valve Dis* 2006;**15**:261-8.
- [28] Otto CM, Burwash IG, Legget ME, et al. Prospective study of asymptomatic valvular aortic stenosis. Clinical, echocardiographic, and exercise predictors of outcome. *Circulation* 1997;**95**:2262-70.
- [29] Novaro GM, Katz R, Aviles RJ, et al. Clinical factors, but not C-reactive protein, predict progression of calcific aortic-valve disease: the Cardiovascular Health Study. *J Am Coll Cardiol* 2007;**50**:1992-8.
- [30] Wierzbicki AS, Viljoen A, Chambers JB. Aortic stenosis and lipids: does intervention work? *Curr Opin Cardiol* 2010;**25**:379-84.

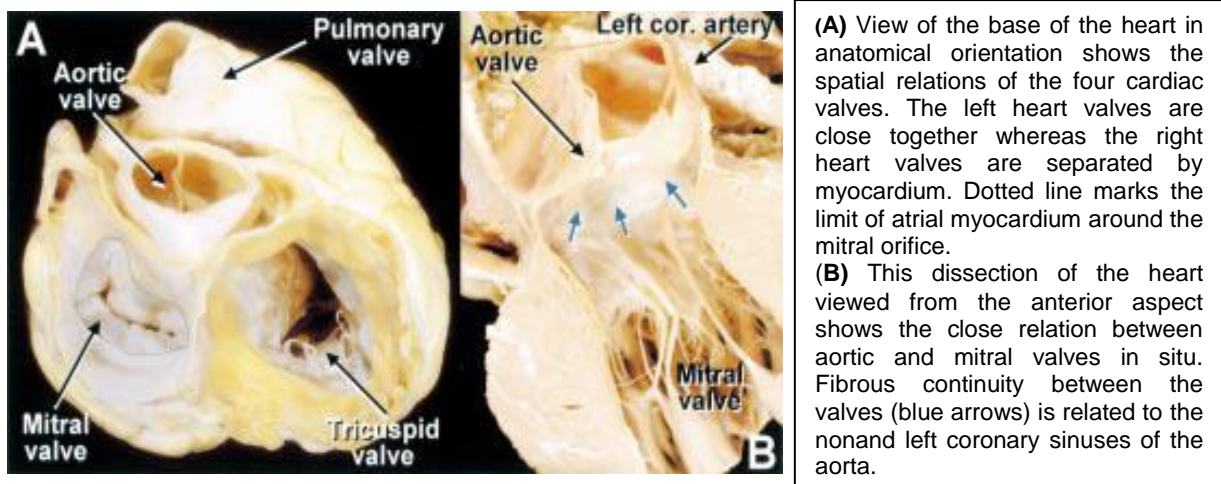


### 3. Mitral valve prolapse: molecular mechanisms and targeted preventive therapies

#### 3.1 Anatomy of the mitral valve

It was Andreas Vesalius who suggested the picturesque term “mitral” to describe the left atrioventricular valve owing to its resemblance to a plan view of the bishop’s mitre.

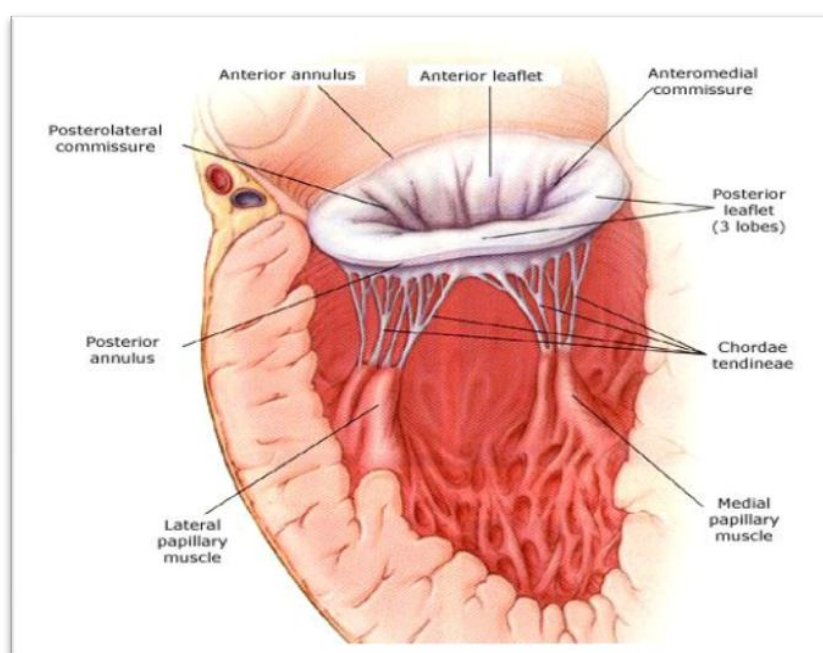
The valvar complex comprises the annulus, the leaflets, the tendinous cords, and the papillary muscles. Also important for its functioning is the left atrial musculature inserting to the leaflets and the myocardium to which the papillary muscles are inserted. The valve is obliquely located in the heart and has a close relation to the aortic valve.



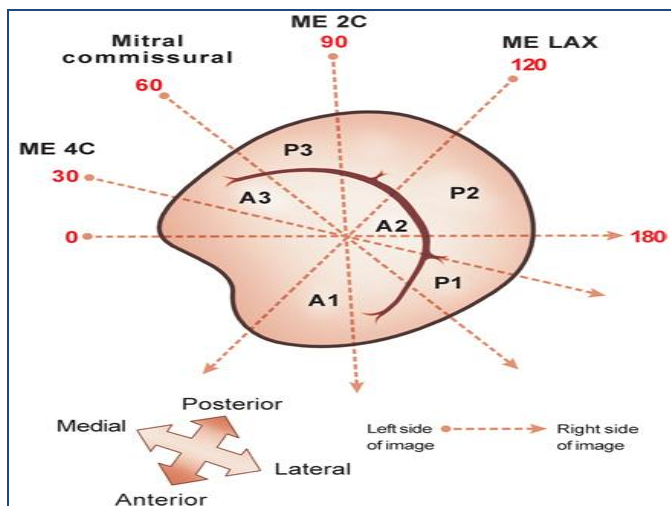
The annulus marking the hingeline of the valvar leaflets is more D shaped than the circular shape portrayed by prosthetic valves. The straight border accommodates the aortic valve allowing the latter to be wedged between the ventricular septum and the mitral valve. In this region, the aortic valve is in fibrous continuity with one of the two leaflets of the mitral valve. Expansions of fibrous tissues at either extreme of the area of continuity form the right and left fibrous trigones.

The atrioventricular conduction bundle passes through the right fibrous trigone. The annulus opposite the area of valvar fibrous continuity tends to be “weaker” in terms of lacking a well formed fibrous cord. This is the area affected in “annular dilation” and also most often involved in calcification of the The annulus opposite the area of valvar fibrous continuity tends to be “weaker” in terms of lacking a well formed fibrous cord. This is the area affected in “annular dilation” and also most often involved in calcification of the annulus. With severe dilation, the minor axis of the valvar orifice becomes so distended that the leaflets, which are of fixed lengths, become unable to approximate each other.

Distinctly different from the tricuspid valve, the mitral valve has two leaflets although some may argue that it has four leaflets. The corresponding terms for anterior and posterior are “aortic” and “mural”. The aortic leaflet hangs like a curtain between the left ventricular inflow and outflow tracts. When the valve is closed, this leaflet appears to form the greater part of the atrial floor but is approximately equal in area to the mural leaflet. With the leaflets meeting, the view of the valve from the atrium resembles a smile. Each end of the closure line is referred to as a commissure. These are designated the *anterolateral* and *posteromedial commissures*.



The free edge of the mural leaflet is often divided into three or more scallops or segments described as lateral, middle, and medial or assigned terms like P1, P2, and P3. Nearer the free edge, the atrial surface is irregular with nodular thickenings. This is also the thickest part, corresponding with the line of closure and the free margin.



Carpentier's classification of the scallops of the mitral valve leaflets

*Tendinous cords* attach to the underside of this area described as the leaflet's rough zone. The rough zone is broadest at the lowest portions of each leaflet but tapers toward the periphery, or commissure, of the closure line. The basal zone that is found only in the mural leaflet is the proximal area that has insertions of basal cords to its ventricular surface. Being distant from the ventricular wall, the aortic leaflet does not have attachments to basal cords. In normal valve closure, the two leaflets meet each other snugly with the rough zone and free edge in apposition but at an angle to the smooth zone. The tendinous cords are string-like structures that attach the ventricular surface or the free edge of the leaflets to the papillary muscles. Characteristically, the tricuspid valve has cordal attachments to the ventricular septum allowing it to be distinguished from the mitral valve on cross sectional echocardiography. The tendinous cords of the mitral valve are attached to two groups of papillary muscles or directly to the postero-inferior ventricular wall to form the tensor apparatus of the valve. Cords that arise from the apices of the papillary muscles attach to both aortic and mural leaflets of the

valve. Since cords usually branch distal to their muscular origins, there are five times as many cords attached to the leaflets as to the papillary muscles.

*Papillary muscles* are the muscular components of the mitral apparatus. As a functional unit, the papillary muscle includes a portion of the adjacent left ventricular wall. Tendinous cords arise from the tips of the papillary muscles. Alterations in the size and shape of the left ventricle can distort the locations of the papillary muscles, resulting in valvar function being disturbed. The papillary muscles normally arise from the apical and middle thirds of the left ventricular wall. Described in most textbooks as two in number, however, there are usually groups of papillary muscles arranged fairly close together. At their bases, the muscles sometimes fuse or have bridges of muscular or fibrous continuity before attaching to the ventricular wall. Extreme fusion results in parachute malformation with potential for valvar stenosis.

### **3.2 Pathophysiology of mitral regurgitation**

Mitral regurgitation is defined as systolic retrograde flow from the left ventricle into the left atrium. All lesions that cause mitral regurgitation do so by reduction or elimination of the normal systolic coaptation between anterior and posterior mitral leaflets, which normally ensures mitral competence. Mechanisms are grossly classified as functional (mitral valve is structurally normal and disease results from valve deformation caused by ventricular remodelling) or organic (intrinsic valve lesions). They can be subclassified by leaflet movement (*Carpentier's classification*): type I (normal valve movement, such as annular dilatation or leaflet perforation); type II (excessive movement); and type III (restrictive movement: IIIa diastolic restriction such as rheumatic disease; IIIb systolic restriction as in functional disease).

Organic			Functional
	Type I*	Type II†	Type IIIa‡
Non-ischaemic	Endocarditis (perforation); degenerative (annular calcification); congenital (cleft leaflet)	Degenerative (billowing/flail leaflets); endocarditis (ruptured chordae); traumatic (ruptured Chord/PM); rheumatic (acute RF)	Rheumatic (chronic RF); iatrogenic (radiation/drug); inflammatory (lupus/anticardiolipin, eosinophilic endocardial disease, endomyocardial fibrosis)
Ischaemic	..	Ruptured PM	..
			Functional ischaemic

MR=mitral regurgitation. PM=papillary muscle. RF=rheumatic fever. \*Mechanism involves normal leaflet movement. †Mechanism involves excessive valve movement.  
‡Restricted valve movement, IIIa in diastole, IIIb in systole.

### Causes and mechanisms of mitral valve regurgitation

Major causes of surgical mitral regurgitation in Western countries are degenerative (primary myxomatous disease, primary flail leaflets, annular calcification), representing 60–70% of cases, followed by ischaemic mitral regurgitation (20%), endocarditis (2–5%), rheumatic (2–5%), and miscellaneous causes (cardiomyopathies, inflammatory diseases, drug-induced, traumatic, congenital). Ischaemic disease probably represents a large proportion of the non-surgical disease burden.

Degenerative mitral regurgitation is usually related to mitral-valve prolapse and rarely to isolated mitral annular calcification. The main phenotypes of mitral prolapse are diffuse myxomatous degeneration (mitral-valve prolapse syndrome or Barlow's disease, sometimes with posterior annular translocation into left atrium) or primary flail leaflets with ruptured chordae affecting the posterior leaflet in 70% of cases, and accompanied by myxomatous degeneration localised to the flail segment and generally normal valve morphology elsewhere. The ischaemic form of this disease rarely results from an organic mechanism (papillary-muscle rupture) and is rarely acute.

Papillary muscle dysfunction plays little part in the generation of functional mitral regurgitation, which is mostly caused by apical and inferior-papillary-muscle displacement due to ischaemic left-ventricular remodelling. Post inflammatory and postradiation mitral regurgitations have similar mechanisms. Retraction of tissue is a major limitation to

successful valve repair. Endocarditic mitral regurgitation might be caused by ruptured chordae or perforations. In all causes, annular enlargement is common, is located mostly or exclusively on the posterior part of the annular circumference, and surgical repair almost always requires annuloplasty.

Physiology of functional mitral regurgitation is even more complex than that of organic mitral regurgitation since ventricular dysfunction predates the regurgitation. Nevertheless, functional mitral regurgitation further increases atrial pressure, which leads to pulmonary hypertension and heart failure. Progression or recurrence after annuloplasty is weakly related to annular enlargement but strongly to increased mitral tenting caused by ventricular remodelling, papillary-muscle displacement and increased chordal traction; however, rates of progression are unknown.

In view of the experimental nature of medical and interventional treatments for mitral regurgitation, surgery is the only treatment recommended by management guidelines. The benefit of early surgery (ie, valve repair in asymptomatic patients) versus a watchful wait was suggested in observational studies but is controversial. Patients with organic mitral regurgitation who have developed severe symptoms (class III or IV), heart failure, or signs of overt left-ventricular dysfunction (ejection fraction <60% or end-systolic dimension  $\geq 40$ –45 mm) have an immediate high risk and therefore prompt surgery—repair (preferable) or replacement is indicated. Even with advanced heart failure or ventricular dysfunction, contraindications to surgery are rare as long as mitral regurgitation remains severe, emphasising the importance of quantitative assessment of disease. Such rescue surgery is indispensable, but is not the preferred timing for surgery in organic disease. Indeed, patients who need to be operated on at such a late stage of their disease have increased mortality after surgery. This outcome emphasises the importance of early detection and assessment of mitral regurgitation. In functional regurgitation, rescue surgery is the most frequent surgical

indication, but consideration should be given to surgery in symptomatic patients before heart failure becomes intractable.

Patients with no or minimum symptoms at baseline cannot expect substantial symptomatic improvement. Those with functional mitral regurgitation are rarely candidates for restorative surgery while asymptomatic but might be suitable for valve repair if coronary artery bypass grafting is necessary independently of the mitral regurgitation. In organic regurgitation, postoperative outcome studies in patients with no or minimum symptoms before surgery show restoration of life expectancy, emphasising the importance of this approach. Patients who are asymptomatic but had either reduced functional capacity by objective exercise testing, hormonal activation, or paroxysmal atrial fibrillation are specific but not exclusive candidates for restorative surgery.

To ensure success of such restorative surgery, important requirements form the basis of advanced mitral-valve repair centres.

## REFERENCES

- [1] Walmsley T. The heart. In: Sharpey-Schafer E, Symington J, Bryce TH, eds. Quain's elements of anatomy, 11th ed, vol 4, pt 3. London: Longmans, Greens & Co, 1929:42.
- [2] Perloff JK, Roberts WC. The mitral valve apparatus. Functional anatomy of mitral regurgitation. *Circulation* 1972;46:227–393.
- [3] Levy MJ, Edwards JE. Anatomy of mitral insufficiency. *Progr Cardiovasc Dis* 1962;5:119–44.
- [4] McAlpine WA. Heart and coronary arteries. An anatomical atlas for clinical diagnosis, radiological investigation and surgical treatment. New York: Springer-Verlag, 1975:39–56.
- [5] Angelini A, Ho SY, Anderson RH, et al. A histological study of the atrioventricular junction in hearts with normal and prolapsed leaflets of the mitral valve. *Br Heart J* 1988;59:712–6.
- [6] Zimmerman J. The functional and surgical anatomy of the heart. *Ann R Coll Surg Engl* 1966;39:348–66.
- [7] Yacoub M. Anatomy of the mitral valve, chordae and cusps. In: Kalmanson D ed. The mitral valve, London: Edward Arnold, 1976:15–20.
- [8] Carpentier A, Deloch A, Dauptain J, et al. A new reconstructive operation for correction of mitral and tricuspid insufficiency. *J Thorac Cardiovasc Surg* 1971;61:1–13.
- [9] Klein A, Burstow D, Tajik A, et al. Age-related prevalence of valvular regurgitation in normal subjects: a comprehensive color flow examination of 118 volunteers. *J Am Soc Echo* 1990; 3: 54–63.
- [10] Nkomo VT, Gardin JM, Skelton TN, Gottdiener JS, Scott CG, Enriquez-Sarano M. Burden of valvular heart diseases: a population-based study. *Lancet* 2006; 368: 1005–11.
- [11] Iung B, Baron G, Butchart EG, et al. A prospective survey of patients with valvular heart disease in Europe: the Euro heart survey on valvular heart disease. *Eur Heart J* 2003; 24: 1231–43.
- [12] Essop MR, Nkomo VT. Rheumatic and nonrheumatic valvular heart disease: epidemiology, management, and prevention in Africa. *Circulation* 2005; 112: 3584–91.
- [13] Zoghbi WA, Enriquez-Sarano M, Foster E, et al. Evaluation of the severity of native valvular regurgitation with two-dimensional and doppler echocardiography: a report from the Task Force on Valvular Regurgitation of the American Society of Echocardiography. *J Am Soc Echocardiogr* 2003; 16: 777–802.
- [14] Bonow RO, Carabello BA, Kanu C, et al. ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons. *Circulation* 2006; 114: e84–231.
- [15] S Y Ho: Anatomy of the mitral valve. *Heart* 2002; iv5-iv 10.



### 3.3 Biology of mitral valve prolapse: the harvest is big, but the workers are few

#### INTRODUCTION

Mitral valve prolapse, i.e. abnormal systolic protrusion of mitral valve leaflets into the left atrium, represents a common cause of severe mitral regurgitation (MR) and it often requires surgical correction, especially in people living in industrialized countries [1,2]. Its average prevalence in the adult population is 2% to 8%; thus, MVP is expected to occur somehow in the life course in approximately 7.2 million individuals in the United States and in over 144 million worldwide [3]. Besides, a marked effect on its spread and severity is played by aging [4]: for instance, important mitral anatomical changes are found at post-mortem in approximately 5-7% of elderly people, with males showing about twice the risk of females of developing severe regurgitation when ageing [5]. With regard to younger people, some studies show that both genders are equally affected, whereas others document a female preponderance [6].

At difference from what happens in chronic ischemic mitral regurgitation (which is a secondary pathologic entity resulting from a variable combination of infarction-induced subvalvular remodeling with subsequent leaflet tethering and annulus dilatation/flattening) [7,8], this disease is, in the majority of cases, a primary condition characterized by a progressive myxomatous degeneration of the mitral valve leaflets and of chordae tendinae [1,2,9]; it represents a slowly developing process which usually shows a benign course, as less than 10% of all prolapsing mitral valves progress to severe regurgitation requiring surgery during their lifetime [10]. Nevertheless, sometimes, people affected by worsening mitral regurgitation suffer from a series of symptoms most represented by dyspnoea and palpitations related to secondary onset of supraventricular tachyarrhythmias, conditions that usually require repeated hospitalization and different diagnostic tests execution. Given the

wide diffusion of MVP, this translates into very high costs for health organizations in the adult population, even if only a small portion of patients will eventually need surgical treatment.

Although clinical features and pathophysiology of MVP are known since several decades, limited data are available regarding biological mechanisms or biochemical perturbations possibly implicated in the progression of this valve disease.

The present study is aimed at summarizing the current knowledge (including preclinical and clinical evidence) about molecular and biological bases of mitral valve myxomatous degenerative disease in order to better understand the continuum of the process and to identify potential therapeutic targets and preventive approaches.

*We will also focus on the description of some biochemical alterations primarily highlighted in patients affected by MVP who present with a cohort of symptoms (only partially shared with simple MVP) that are not directly related to the hemodynamics of mitral regurgitation. This condition, known as mitral valve prolapsed syndrome (MVPS), is characterized by chest pain, dysrhythmia, anxiety, insomnia and syncope [11], and is associated with a certain degree of adrenergic and renin angiotensin aldosterone (RAA) system impairment, a condition showing only partially common features with pure-isolated MVP disease.*

## GENETIC

Several studies have shown that MVP can be familial, sporadic, or can occur secondary to a variety of connective tissue disorders such as Ehlers-Danlos syndrome, Sticker syndrome and adult polycystic kidney disease [11]. Also, in recent years there has been a significant effort in identifying genetic profiles related to MVP, especially through the use of linkage analysis, in order to define a genetic screening tool available for clinicians. These studies, however, have identified a specific subset of familial primary MVP, whose inheritance appears to be attributable to an autosomal dominant mode with variable expression [12]. Familial genetic

linkage studies were performed with the aim of mapping possible loci for MVP occurrence, and have shown that several genes located on chromosomes 11,13,16 and X (some of them being partially related to human leukocyte antigens, HLA) have a weak association with the familial form of this disease (12), with the possible exception of the locus MMVP3 on chromosome 13 which is strongly associated with the familial variant of MVP [13] and of the X-chromosome-gene coding for filamin A (a cytoskeleton protein) which seems to be responsible for X-linked form of Barlow's disease [14]. The possibility, however, that these preliminary data can be extrapolated beyond the context of the rare entity of familial MVP has not yet been verified.

An alternative hypothesis, which considers the somehow obvious concept that familial MVP arises from a heritable collagen gene defect, has been tested through the analysis of its segregation pattern in affected families [15]. In contrast to what expected, the trait was shown not to be linked to COL1A1, COL1A2, COL3A1 or COL5A2 loci, which suggests that valve abnormalities of familial MVP are not caused by a structural defect in any of these genes.

Interestingly, a more definite role seems to be played by specific mutations in the genes for Transforming Growth Factor Receptor I and II (TGFBRI), which appear to be associated with particular phenotypic expression and cardiovascular (including MVP) and skeletal features in the context of general connective tissue disorders which are only partially shared with the classic form of Marfan Syndrome, thus allowing the proposition of new disease entities named Marfan type II, Loeys-Dietz, Shprintzen-Goldberg and Furlong syndromes [16]. Again, the real importance of TGFBRI in the development of sporadic MVP beyond the Marfan-like milieu needs further evaluation.

In addition, several studies have tried to assess possible links between a variety of gene polymorphisms and sporadic non familial MVP, focusing on genetic factors implicated in extracellular matrix remodeling, collagen metabolism, RAA system and haemostasis.

Concerning collagen metabolism, in three different studies Chou et al. have addressed both genotype distribution and allelic frequencies of these genes in one hundred MVP patients compared to, respectively, 140 [17], 243 [18], and 106 [19] age and sex – matched normal control subjects. These studies show a significant association between sporadic MVP onset and certain genotypes of fibrillin-1 gene (exon 15 TT and 27 GG), of collagen (COL3A1 exon 31 GG) [17,18] and of urokinase – plasminogen activator (PLAU) (a protein-system acting as a trigger in initiating metalloproteinases pathway for extracellular matrix degradation in aortic aneurysms and consequently perhaps involved in collagen turnover). This observation results in partial contrast with previous findings concerning familial MVP [16] that did not show any role of these genes in disease occurrence. In addition, the significant allelic frequency difference found (in particular regarding COL3A1 exon 31 polymorphism, whose p-value was highly significant) suggests a pivotal role in MVP occurrence and progression, since these allelic variants render fibrillin 1 and collagen less resistant and more extensible, similarly to what happens in connective tissue disorders such as Marfan and Ehlers-Danlos syndromes [17,18,19].

Unfortunately, all these associations derive from studies carried on geographically and statistically limited population subsets, leaving still open the question whether they could have a general meaning.

In addition to collagen strength and resistance to fatigue, the attention of investigators pointed on the assessment of the possible role of some genetic characters in accelerating mitral valve disease progression, suggesting that matrix metalloproteinases-3 (MMP-3) promoter haplotype may represent a marker of adverse and rapid pathologic course, leading to fast increasing valve regurgitation and to consequent left ventricular remodeling process [20].

Finally, the role of RAA system has been explored, especially in patients affected by the additional symptoms of MVPS, through the assessment of the existence of a genetic link

between MVP and a higher allelic frequency of angiotensin converting enzyme (ACE) II genotype [21] and of A-C (1166) polymorphism of the angiotensin II type 1 receptor gene [22]. Specifically, the presence of ACE II genotype provides an Odd-Ratio (OR) for MVP onset risk of 2.14, whereas the OR in favor of carrying the C1166 allele of angiotensin-1 (AT1) receptor is four times greater for MVP patients than for controls. All these findings are consistent with the hypothesis that ACE system is involved in the occurrence/progression of this disease, as documented both in Asiatic and European populations. The contribution of other neuroendocrine perturbations still needs further investigation.

In conclusion, both the genes involved in tissue strength and in tissue remodeling may sensibly affect MVP occurrence; RAA system also seems to play a key role, especially in the context of MVP syndrome. Further validation from additional studies is warranted, especially because genetic data have been obtained in relatively limited numbers of patients.

## **HAEMOSTASIS AND PLATELET FUNCTION**

The clinical observation of an higher incidence of thromboembolic events of unidentified origin in patients affected by MVP with respect to general population has been documented since 1980 by Barnett et al. [23], with a particular emphasis on the occurrence of repeated episodes of cerebral ischemia in specific subsets of MVP patients who, for unknown reasons, are greatly predisposed to these body weakening complications. Although available evidence in favor of the existence of a substantial link between MVP and thromboembolism is strong [24-27], some Authors are critical with these findings [28]; in addition, epidemiologic studies have raised several questions on the association between MVP and cerebro-vascular complications documenting that stroke incidence in young patients seems to be unrelated to the echocardiographic diagnosis of mitral leaflet prolapse when the degree of valve insufficiency is not relevant [29] and that MVP patients with angiographically normal

coronary vessels fail to show a pro-thrombotic status [30]. This is –however- somehow in contrast with several other studies, which support an association between MVP and perturbations of the haemostatic pathways, although evidence in favor and against this association is available in literature [24-28,31,32,33,37,39].

MVP disease has been associated with some degree of platelet and haemostatic abnormalities in the early eighties [31,32,33], but a consensus on the main haemostatic perturbations associated with this disease was never reached; also, it is unclear whether MVP represents the cause or the effect of these abnormalities. Some Authors documented a slightly augmented platelet activation (assessed both in plasma before and after stimulation of platelets with conventional agonists by measuring beta-thromboglobulin and platelet factor 4 [25,34], whereas Others have shown a somehow reduced platelet activation, as evidenced by the expression of P-selectin after stimulation of platelets [24]; in addition, an increase in thrombin generation has also been found in patients affected by MVP [24]. Interestingly, a higher degree of valve insufficiency was associated with more marked haemostatic perturbations, suggesting that the disease per se is not responsible for the activation of platelets, which is, otherwise, secondary to the altered patients' haemodynamics (whose entity is directly proportional to the increase of regurgitant blood fraction) or to abnormalities occurring at the platelet level directly on the surface of diseased valves [24]. It should be mentioned, however, that other studies reached completely opposite conclusions, e.g. no systemic beta-thromboglobulin increase in neurologically asymptomatic MVP patients [35] nor major platelet function alterations [36], with the only exception of impaired aggregation [37], consisting in a slight decrease of basal platelet aggregation rate in a relatively limited number of patients.

It has also been reported that about one third of asymptomatic patients shows increased levels of platelet factor-4 [34] or of beta-thromboglobulin levels [36], as well as increases in

von Willebrand factor (vWf) antigen or fibrinopeptide A [37]. Even if no clinical study provides evidence for this hypothesis, we can envision that also in patients who did not experience thromboembolic complications, there might be a subgroup at highest risk for these complications. Walsh et al. documented that the increased platelet coagulant activity (e.g. the ability of platelets to activate factor XII thus promoting the interaction among factors XIa, VIII, IX and X, leading to the initiation of coagulation) and the augmented proportion of circulating platelet aggregates occur concomitantly with clinical thromboembolic phenomena, leading to transient visual obscurations and retinal or cerebral ischemic attacks [26]; even platelet survival time [27] and global fibrinolytic capacity [38], which provides information of the global fibrinolytic system degree of activity, are significantly shortened in those patients with MVP developing systemic embolism or presenting an history of transient ischemic attacks.

In one trial the fibrinolytic system has been assessed in MVP patients in comparison with individuals affected by nonrheumatic calcific aortic valve stenosis. In this study, higher plasma fibrinogen levels were found in both groups of patients when compared to “normal” population values. Instead, mitral patients presented lower fibrin D-dimer plasmatic concentrations than aortic and control subjects; these findings seem to be consistent with the existence of lesser intravascular clotting in MVP with respect to calcific aortic stenosis and, consequently, with different mechanisms implicated in clinical thrombogenesis [39].

A certain degree of common rheologic similarities involving aortic and mitral diseases is, however, further supported by Francis, who investigated platelet aggregation in response to epinephrine and ADP stimuli in patients undergoing valvular heart surgery in comparison to patients with coronary artery bypass grafting (CABG): interestingly, abnormal ADP-related occlusion is more frequent in valvular patients, reflecting a pre-existing high-shear damage to platelets that renders them refractory to subsequent shear activation and aggregation [40].

Again, this phenomenon appears to be the consequence, rather than the cause, of MPV. In partial contrast with previous data are the results reported by Olsen et al. who studied haemostatic variables in the main canine model of MVP pathology, referring a form of platelet dysfunction identified at high shear rates regardless of mitral regurgitation gravity and associated to a qualitative vWF defect; at variance to what reported for human subjects, dogs with mitral regurgitation had enhanced platelet aggregation compared to controls [40,41]. Interestingly, in a previous study, the same Authors performed a subgroup analysis stratifying animals according to the degree of valve insufficiency: they reported an increase in circulating fibrinogen concentration (directly related to left ventricular end diastolic diameter and left atrial to aortic root ratio) among all dogs, and they also observed that the severity of mitral regurgitation was proportionally associated with low plasma vWF concentration, most likely due to the loss of vWF high molecular weight multimers (HMWMs) [42]. This, however, needs to be confirmed in humans. In this context, it is worth mentioning that a similar decrease in the circulating levels of HMWMs of vWF was reported in patients affected by aortic stenosis due to the action of ADAMTS-13, a matrix metalloproteinase that acts on vWF preferentially under conditions of high shear stress, as it happens through a stenotic valve orifice or –possibly- in case of regurgitant mitral valves. In the context of calcific aortic stenosis, such phenomenon may lead to significant bleeding episodes (often classified as Heyde's syndrome), which are also documented in a minority of MVP patients [33,43,44], which is suggestive for a common underlying pathogenetic mechanism. The reasons why in MVP haemostatic alterations preferentially result in thromboembolic rather than bleeding events, as it occurs in aortic stenosis, are not known. Finally, Girolami et al. documented that some rare congenital clotting defects (namely the combined phenotypic deficiency of FX and of FVII) are accompanied by a higher percentage, with respect to



general population, of a cohort of haemostasis-unrelated abnormalities, including mitral myxomatous degeneration [45].

In conclusion, currently available data suggest possible links between MVP and perturbations of the clotting and fibrinolytic system and of platelet function as well, which are potentially linked to thromboembolic complications occurring in this disease. Evidence currently available, however, is confined to some animal studies or to studies carried out many years ago, a feature limiting their immediate translation into clinical practice. Finally, another important issue concerns the timing of the occurrence of these perturbations, as it is unclear if they anticipate or follow MVP onset.

## **INFLAMMATION**

The potential role of inflammatory processes in relation with MVP onset has seldom been studied; only one recent study [46], carried out in six mitral nonrheumatic prolapsing valves specimens obtained from patients undergoing mitral valve repair, addressed apolipoproteins and inflammatory cells detection in this disease. In comparison to stenotic aortic valves, both mitral and aortic regurgitant valves showed a significant lower degree of inflammatory infiltrates (composed by lymphocytes positive for CD3, CD20 and CD68 antigens and macrophages) and of apolipoprotein A-1 and B deposition. These preliminary findings suggest that, at variance with what happens in aortic stenosis onset and progression [47], inflammation and cholesterol metabolism may not be the main actors in the course of this pathology. Further studies, however, are needed to confirm this hypothesis.

## **OXIDATIVE STRESS AND ENDOTHELIAL FUNCTION**

The hypothesis that a primitive increase of oxidative stress goes along with MVP development was first tested several years ago by Arocha et al. who studied the production of malondialdehyde (MDA) in forty asymptomatic patients who were found by chance to be

affected by myxomatous mitral disease at echocardiography [36]. They documented significantly augmented plasma MDA levels in MVP patients with respect to a control group of 17 age and sex matched controls. Animal studies also show that a certain degree of oxidative state impairment occurs, detected both at a systemic and at a local level; in particular, there is an increased NADPH-diaphorase activity and subsequent augmented nitric oxide synthase (NOS) expression in areas of mitral valve showing myxomatous changes [48] and decreased plasmatic Nox concentrations directly proportional to the severity of mitral insufficiency [49]. This suggests that nitric oxide (NO) may play a role in MVP pathogenesis; in addition, endothelial dysfunction may also develop early in the course of valve disease, and it seems to worsen as regurgitation becomes more relevant. This has been further confirmed by the finding of augmented NO release in regions of early prolapsing porcine mitral valve leaflets presenting accumulation of mucopolysaccharide deposits and defraction of the valve structure (and consequently more prone to myxomatous degeneration and prolapse), thus suggesting local changes of both endothelial and inducible NOS expression [50]. Moreover, another study collecting postmortem canine mitral valve specimens with clear macroscopic signs of myxomatous degeneration showed that diseased areas with collagen degeneration and fibrosis had higher endothelin-receptor spreading, whose density within, as well as, on the mitral leaflets was significantly associated with the degree of myxomatous disease [51].

Unfortunately, all available data, with the exception of the study by Arocha, concern pre-clinical MVP models, which consequently still need confirmation in humans. The increase in oxidative stress, however, is not a peculiarity of MVP; in fact, in nonrheumatic stenotic aortic valve disease the markers of oxidative stress are increased within the valve tissue [47]; and a recent study by Rabus et al. suggests that systemic oxidative stress increase can be found both in aortic and mitral valve disease, of both rheumatic and nonrheumatic etiology [52].

These data highlight a potential role, although probably not disease-specific, of both oxidative stress and endothelial dysfunction in MVP, requiring additional substantiation from future studies; finally, even in this case, the question if the activation of these pathways is causative or not is still open.

## **EXTRACELLULAR MATRIX REMODELING AND BONE METABOLISM**

It is recognized that myxomatous degeneration is the pathological substrate of MVP, whose main feature is represented by redundant leaflets; floppy valves likely result from mechanically inadequate extracellular matrix (ECM) status, particularly collagen, thereby allowing stretching of the leaflets [5,53]. More in detail, at the ultrastructural level, several studies have shown fragmentation, splitting, swelling and coarse granularity of some collagen fibrils and a spiraling twisted appearance of others in diseased valves [54,55,56,57]; changes in elastic fibers, including an increase in the content of elastic tissue, have also been reported [58].

Nevertheless, the real mechanisms responsible for the ECM defect and/or reparative processes that strengthen its faulty status are still uncertain. Basing upon the hypothesis that, as in other tissues, ECM turnover depends on a dynamic balance between synthesis and degradation that occurs through the action of matrix metalloproteinases (MMP) and cysteine endoproteases, Rabkin et al. evaluated the role of valvular interstitial cells and catabolic enzymes in the pathogenesis of myxomatous degeneration, documenting that myxomatous interstitial cells show features of activated myofibroblasts expressing higher levels of collagenases (MMP-1, MMP-13), gelatinases (MMP-2, MMP3), and other proteolytic enzymes (cathepsin S and K) when compared with analogous normal mitral valves obtained at autopsy [59]. The role of altered ECM turnover and metabolism in determining leaflets prolapse was later confirmed by another study [60] comparing molecular and histological features in normal and ruptured chordae tendinae cordis (CTC) conditioning severe mitral regurgitation: in particular, the

Authors pointed their attention on the differential local expression of tenomodulin (a recently isolated anti-angiogenic factor) measured in various areas of CTC. They showed that tenomodulin is locally absent in the ruptured zones of chordae, favoring abnormal vessel formation also in combination with enhanced expression of vascular endothelial growth factor-A (VEGF-A). Moreover, differently from what observed in normal or non-ruptured areas, higher numbers of inflammatory cells positive for CD11b, CD14 and vimentine and with an augmented expression of MMP-2 and 13 were detected in association to the downregulation of tenomodulin. The role of tenomodulin and of the different pathways related to this molecule could be of great clinical interest, since we have to keep in mind that the crucial passage from simple elongated chordae to their rupture frequently coincide with symptoms onset and consequently with a probable surgical indication.

Interestingly, changes in metalloproteinase expression was also found in specimens of left and right appendages taken from patients undergoing mitral valve surgery, which showed downregulation of MMP-1 and -9 with respect to patients undergoing CABG; at variance, MMP-2 and TIMP-1, -2 and -4 expression were similar. This suggests that, although sensibly different in terms of activation or repression, the involvement of metalloproteinases in MVP is not limited to the mitral valve but can also occur in other heart structures [61].

Moreover, the influence of interstitial cells constituting valve ECM on MVP onset and development has also been documented in some animal models and in human specimens: in fact, mesenchymal cell activation ( $\alpha$ -SMA positive cells overexpression) [62] and phenotype transformation (towards CD34+ fibrocytes, [63] occur throughout the valve leading to dramatic cellularity increase and metalloproteinases secretion (MMP-2 and -9), whose degree of expression directly correlates with disease severity [64]. In addition, the role of CD34+ fibrocytes is of particular interest; this cell population, in fact, is able to synthesize MMP-9 as well as collagen I and III (constituting a crucial factor in MVP pathogenesis) and seems to be

responsible for tissue repair subsequent to mechanical alteration of the mitral valve [63]. Cellular proliferation, associated to tissue redundancy, appears to be augmented even in genetically engineered mice resembling patients affected by Marfan Syndrome and MVP, thus providing critical insight into the pathogenetic mechanism of such abnormalities in syndromic and perhaps non-syndromic variants of mitral valve disease [65]. In particular, Ng et al documented that mitral valves isolated from fibrillin-I deficient mice exhibit post-natally acquired alterations in ECM architecture secondary to augmented cell proliferation, decreased apoptosis and excess Transforming Growth Factor-  $\beta$  (TGF- $\beta$ ) activation and signaling. Numerous TGF- $\beta$  related genes, such as  $\beta$ IGH3, endothelin I and tissue inhibitor of MMP-1 (TIMP 1) resulted overexpressed; since they are all involved in cellular proliferation and survival, it is plausible to hypothesize their relevant contribution to myxomatous degeneration development [65].

Once more, the issue whether such phenomena play a consistent role even in humans is still open.

Only recently, mitral valve degeneration has been associated to an osteoblastic differentiation process mediated by the low-density lipoprotein receptor-related protein 5 signaling pathway that might cause leaflet thickening. In fact, Caira et al have highlighted some common features that characterize two frequent valvular pathologies (aortic stenosis and MVP), since an endochondral bone differentiation is evident in both, but it is expressed as cartilage in mitral valves and as pure bone in the aortic ones [66]. Such conclusion is also consistent with the crude “in vivo” aspect of valve leaflets inspected at surgery, as the stenotic aortic ones are often fused and deeply calcified, while mitral ones seem fibroelastic “entities” flopping into the left atrium.

Taken together, currently available evidence suggests that CTC rupture is not accidental, but can occur when and where CTC are weakened by neoangiogenesis, MMP activation and

infiltration of inflammatory cells secondary to the loss of or damage to the tenomodulin layer, a phenomenon perhaps subsequent to locally increased mechanical stress or to primitive inflammatory events.

## **MVP AND MVPS: A COMMON BIOLOGIC BACKGROUND ?**

As stated before, a subgroup of patients affected by MVP presents a number of complaints that seem to be completely independent from their primary cardiac disease, establishing the so-called mitral valve prolapse syndrome. Typical clinical manifestations of this pathology include unexplained chest pain (not related to atherosclerosis or to other evident causes), palpitations, fatigue, exercise intolerance, insomnia, dizziness and panic attacks which are so relevant to induce psychiatric-like disorders in affected patients. Such symptoms often have a great impact on patients' daily life, probably more than those strictly related to valve dysfunction "per se", since, as usual, they are underestimated or misunderstood by physicians; therefore, we believe that an effort in clarifying the pathogenesis of these complaints and the reason why not all affected people share the same symptoms needs to be performed, considering also the fact that only "spot" evidences are available in medical literature.

In particular, Authors have focused their attention on enhanced adrenergic activity as a possible cause, whereas others have implicated magnesium deficiency or perturbations of the RAA system.

**Renin-angiotensin-aldosterone system.** An impairment of RAA has been found even in the early phases of degenerative mitral disease in dogs, when the animals are still asymptomatic or mildly symptomatic; this excludes the role of heart failure secondary to MV disease in the up-regulation of RAA. In particular, both plasma renin activity (PRA) and aldosterone concentration are significantly higher compared to controls [67], and the levels of

the serum angiotensin-converting enzyme (ACE) activity are also increased [68,69]. The concomitant finding of normal concentrations of endothelin-1, atrial natriuretic peptide and arginine vasopressin, all peptides of pathophysiologic importance in congestive heart failure (CHF), further supports the concept that the valve disease itself, and not its clinical sequelae ultimately leading to heart failure, underlie RAA activation.

In translating similar concepts into “human patients”, many Authors have shown various degrees of RAA impairment in MVPS patients: in detail, it has been shown that these patients display abnormal regulation patterns of RAA during upright posture and volume depletion, consisting in a greater increase of PRA and in the absence of changes in plasma aldosterone levels compared to controls [70,71].

**Adrenergic activity.** The existence of an autonomic dysfunction and of an enhanced adrenergic tone has also been investigated in both isolated MVP and in MVPS. Several Authors have shown abnormal adrenergic reactivity (which may explain the RAA system impairment too), as suggested by the presence, in MVPS patients, of higher 24-hour urinary vanillilmandelic acid (VMA) excretion, of greater resting heart rate in MVPS young men [70] and of higher epinephrine and norepinephrine levels in plasma [71]. Excessive vagal tone and  $\alpha$ -adrenergic hyperactivity have also been documented [72]. Interestingly, similar neuroendocrine alterations have been shown in pure MVP patients without an history of autonomic symptoms. And MVP patients also exhibit a greater incidence of syncope (when compared to controls), more orthostatic hypotension during quiet standing and loss of the normal decrease with age in vagally-mediated heart rate variability during deep breathing. In addition, a lower 24-hour epinephrine excretion was observed, which is suggestive of an hidden autonomic dysfunction not clinically evident associated with the presence “per se” of the cardiac lesion [73].

Finally, evidence is currently available concerning the association between increased adrenergic state and augmented plasmatic values of atrial natriuretic factor in MVPS, especially in hypovolaemic individuals [71,74]; the interplay between these two neuro-endocrine disorders may account for some of the addictive symptoms, by determining the imbalance between sympathetic and parasympathetic system, the altered RAA response to orthostatic stimulus and the volemic and venous flow reductions.

**Hypomagnesemia.** The existence of a strong relation between MVP and hypomagnesemia is a rather old concept clearly assessed both from a veterinary standpoint [69] (tested, once more, in Cavalier King Charles spaniels dogs without evidence of heart failure) and from a clinical one. Studies performed in patients suffering from accessory symptoms of MVPS show that decreased serum magnesium represents a common feature and that supplementation of this ion leads to a substantial improvement in the clinical picture along with the reduction in catecholamine excretion [75]. Additional data indicate that plasma magnesium concentration does not correlate with MVP severity and degree of regurgitation [69]. Instead, it is paralleled by low total intracellular erythrocyte magnesium levels [76].

Taken together, these data suggest that electrolytic unbalance and autonomic dysregulation represent two of the main players in MVP, being a potentially therapeutic target to mitigate auxiliary symptoms onset and perhaps disease progression. A major issue, however, still needs to be addressed. The main question is whether a continuum exists starting from the isolated MVP towards the clinical picture of MVPS and whether this is paralleled by progressively increasing biologic perturbations.



## CONCLUSIONS

At variance with coronary artery disease or with calcific aortic valve stenosis, and in spite of its high prevalence, historically, myxomatous degenerative mitral valve disease has always been ignored or, at best, underestimated from a biological point of view.

We have reviewed here currently available evidence concerning the biological features of MVP. Data show that current knowledge about MVP is limited to somehow isolated spots which suggest a possible role of inflammation, oxidative stress, haemostasis and a potentially prominent role of extracellular matrix remodeling in mitral valve disease genesis and progression. Unfortunately, a deep comprehension of the pathways involved and the certainty whether they represent simple associated elements, real protagonists, true causes or only consequences of the pathology still lack.

On the other hand, these preliminary data are, undoubtedly, a starting point worthy of deeper investigation, which will allow, in the future and if widely confirmed, to identify early pharmacological tools and targeted therapeutic strategies, in order to achieve a slackening of the disease progression or even a slight regression of this probably multifactorial process. Indeed, as, in the majority of cases, there is a long time-gap between MVP diagnosis (often made by chance because of lack of symptoms) and the occurrence of “full maturation” with consequent mitral valve repair need, we can hypothesize that, for patients who receive this diagnosis at an early stage and who are for this reason strictly echocardiographically monitored, a targeted drug therapy with some class of drugs already available (e.g. statins, angiotensin-converting enzyme inhibitors, angiotensin-2 receptors blockers, beta-blockers or anti-proliferative molecules), or with drugs newly developed to this purpose, may be useful. Further prospective studies are eagerly needed to assess if this way is valuable of consideration, but we expect and hope that in a near future more knowledge concerning this

area will be available, as our current surgical therapy of MVP can only mechanically correct the defect of the valve but not the underlying molecular mechanisms.

## REFERENCES

- [1] Devereux RB. Recent developments in the diagnosis and management of mitral valve prolapse. *Curr Opin Cardiol* 1995; 10:107-116.
- [2] Braunwald E. Valvular heart disease. In: Braunwald E, editor, *Heart disease. A textbook of cardiovascular medicine*, 5<sup>th</sup> ed., Philadelphia: W.B. Saunders, 1997:1007-1072.
- [3] Savage DD, Garrison RJ, Devereux RB et al. Mitral valve prolapse in the general population; epidemiologic features: the Framingham Study. *Am Heart J* 1983; 106:571-575.
- [4] Pedersen H, Haggstrom J. Mitral valve prolapse in the dog: a model of mitral valve prolapse in man. *Card Res* 2000; 47:234-243.
- [5] Davies MJ, Moore BP, Braimbridge MV. The floppy mitral valve – Study of incidence, pathology and complications in surgical, necropsy and forensic material. *Br Heart J* 1978; 40:468-481.
- [6] Cheng TO, Barlow JB. Mitral leaflet billowing and prolapse: its prevalence around the world. *Angiology* 1989; 40:77-87.
- [7] Gelsomino S, Lorusso R, Caciolli S et al. Insights on left ventricular and valvular mechanisms of recurrent ischemic mitral regurgitation after restrictive annuloplasty and coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2008; 136:507-518
- [8] Gelsomino S, Lorusso R, Billé G et al. Left ventricular diastolic function after restrictive mitral ring annuloplasty in chronic ischemic mitral regurgitation and its predictive value on outcome and recurrence of regurgitation. *Int J Cardiol* 2009; 132:419-428
- [9] Fontana ME, Sparks EA, Boudoulas H, Wooley CF. Mitral valve prolapse and the mitral valve prolapse syndrome. *Curr Probl Cardiol* 1991; 16:309-375.
- [10] Zuppiroli A, Rinaldi M, Kramer-Fox R, Favilli S, Roman MJ, Devereux RB. Natural history of mitral valve prolapse. *Am J Cardiol* 1995; 75:1028-1032.
- [11] Boudoulas H. Mitral valve prolapse: etiology, clinical presentation and neuroendocrine function. *J Heart Valve Dis* 1992; 1:175-188.
- [12] Grau JB, Pirelli L, Yu P-J, Galloway AC, Ostrer H. The genetics of mitral valve prolapse. *Clin Genet* 2007; 72:288-295.
- [13] Nesta F, Leyne M, Yosefy C et al. New locus for autosomal dominant mitral valve prolapse on chromosome 13: clinical insights from genetic studies. *Circulation* 2005; 112:2022-2030.
- [14] Kyndt F, Le Scouarnec S, Jaafar P et al. Genetic aspects of valvulopathies. *Arch Mal Coeur Vaiss* 2007; 100:1013-1020
- [15] Henney AM, Tsipouras P, Schwartz RC, Child AH, Devereux RB, Leech GJ. Genetic evidence that mutations in the COL1A1, COL1A2, COL3A1 or COL5A2 collagen genes are not responsible for mitral valve prolapse. *Heart* 1989;61:292-299.
- [16] Akutsu K, Morisaki H, Takeshita S et al. Phenotypic heterogeneity of Marfan-like connective tissue disorders associated with mutations in the Transforming Growth Factor Receptor genes. *Circulation J* 2007; 71:1305-1309

- [17] Chou HT, Shi YR, Hsu Y, Tsai FJ. Association between fibrillin-1 gene exon 15 and 27 polymorphisms and risk of mitral valve prolapse. *J Heart Valve Dis* 2003; 12:475-481.
- [18] Chou H, Hung J, Chen Y, Wu J, Tsai F. Association between COL3A1 collagen gene exon 31 polymorphism and risk of floppy mitral valve/mitral valve prolapse. *Int J Cardiol* 2004; 95:299-305.
- [19] Chou H, Chen Y, Wu J, Tsai F. Association between urokinase-plasminogen activator gene T4065C polymorphism and risk of mitral valve prolapse. *Int J Cardiol* 2004; 96:165-170.
- [20] Oceandy D, Yusoff R, Baudoin F, Neyses L, Ray S. Promoter polymorphism of the matrix metalloproteinase 3 gene is associated with regurgitation and left ventricular remodelling in mitral valve prolapse patients. *Eur J Heart Fail* 2007; 9:1010-1017.
- [21] Chou H, Chen Y, Shi Y, Tsai F. Association between angiotensin I-converting enzyme gene insertion/deletion polymorphism and mitral valve prolapse syndrome. *Am Heart J* 2003; 145:169-173.
- [22] Szombathy T, Janoskuti L, Szalai C et al. Angiotensin II type 1 receptor gene polymorphism and mitral valve prolapse syndrome. *Am Heart J* 2000; 139:101-105.
- [23] Barnett HJ, Boughner DR, Taylor DW, Cooper PE, Kostuk WJ, Nichol PM. Further evidence relating mitral valve prolapse to cerebral ischemic events. *N Engl J Med* 1980; 302:139-144.
- [24] Martini F, Zuppiroli A, Gori A et al. Platelet and blood clotting activation in patients with mitral valve prolapse. *Thromb Res* 1996; 83:299-306.
- [25] Tse H, Lau P, Cheng G. Relation between mitral regurgitation and platelet activation. *JACC* 1997; 30:1813-1818.
- [26] Walsh P, Kansu T, Corbett J, Savino P, Goldburgh W, Schatz N. Platelets, thromboembolism and mitral valve prolapse. *Circulation* 1981; 3:552-559.
- [27] Steele P, Weily H, Rainwater J, Vogel R. Platelet survival time and thromboembolism in patients with mitral valve prolapse. *Circulation* 1979; 60:43-45.
- [28] Cheitlin MD. Thromboembolic studies in the patient with prolapsed mitral valve. Has Salome dropped another veil? *Circulation* 1979; 60:46-47.
- [29] Gilon D, Buonando S, Joffe MM et al. Lack of evidence of an association between mitral valve prolapse and stroke in young patients. *N Engl J Med* 1999; 341: 8-13.
- [30] Chevalier JM, Bonnet J, Brottier L et al. Mitral valve prolapse and angina with normal coronary arteries : research of a thrombogenic factor. *Ann Cardiol Angeiol* 1991; 40:419-422.
- [31] Leone G, Valori VM, Sandric S, Cudillo L, Bizzi B. Platelet activation and thromboembolism in patients with mitral valve prolapse. *Thromb Res* 1982; 28:831-835.
- [32] Pickering NJ, Brody JI, Barrett MJ. Von Willebrandt syndromes and mitral valve prolapse; linked mesenchymal disorders. *N Engl J Med* 1981; 305:131-134.
- [33] Gamba G, Venco A, Grandi A et al. Mitral valve prolapse and Factor VIII complex abnormalities. *Haematologica* 1984; 69:549-555.

- [34] Fisher M, Weiner B, Ockene IS, Forsberg A, Duffy CP, Levine PH. Platelet activation and mitral valve prolapse. *Neurology* 1983; 33:384-386.
- [35] Lin SL, Fisher MJ, Tak T, Rahimtoola SH, Chandraratna PA. Platelet activation in patients with mitral valve prolapse. *Can J Cardiol* 1989; 5:84-86.
- [36] Arocha F, Diez-Ewald M, Durango AI, Sulbaran T. Platelet activity in mitral valve prolapse: a study of platelet aggregation, malondialdehyde production and plasma beta-thromboglobulin. *Am J Hematol* 1985; 19:21-25.
- [37] Zuppiroli A, Cecchi F, Ciaccheri M et al. Platelet function and coagulation studies in patients with mitral valve prolapse. *Clin Cardiol* 1986; 9:487-492.
- [38] Atalar E, Acil T, Aytemir K et al. Diminished global fibrinolytic capacity in patient with mitral valve prolapse is associated with transient ischemic attacks. *Clin Appl Thromb Haemost* 2002; 8:41-44.
- [39] Lip GY, Rumley A, Dunn FG, Lowe GD. Thrombogenesis in mitral regurgitation and aortic stenosis. *Angiology* 1996; 47:1117-1125.
- [40] Francis JL. Platelet dysfunction detected at high shear in patients with heart valve disease. *Platelets* 2000; 11:133-136.
- [41] Tarnow I, Kristensen AT, Olsen LH et al. Dogs with heart disease causing turbulent high-velocity blood flow have changes in platelet function and von Willebrandt factor multimer distribution. *J Vet Intern Med* 2005; 19:515-522.
- [42] Tarnow I, Kristensen AT, Olsen LH, Pedersen HD. Assessment of changes in haemostatic markers in Cavalier King Charles Spaniels with myxomatous mitral valve disease. *Am J Vet Res* 2004; 65:1644-1652.
- [43] Pichering NJ, Brody JT, Barrett MJ. Von Willebrandt syndromes and mitral valve prolapse; linked mesenchymal dysplasias. *N Engl J Med* 1981; 305:131-134.
- [44] Fromm B, Margulis T, Grenadier E, Palant A, David M, Aghai E. Von Willebrand factor and mitral valve prolapse. *Thromb Haemost* 1988; 60:230-231.
- [45] Girolami A, Ruzzon E, Tezza F, Scandellari R, Scapin M, Scarpato P. Congenital FX deficiency combined with other clotting defects or with other abnormalities: a critical evaluation of the literature. *Haemophilia* 2008; 14:323-328.
- [46] Lars W, Thora S, Broqvist M. Role of inflammation in nonrheumatic, regurgitant heart valve disease. A comparative, descriptive study regarding apolipoproteins and inflammatory cells in nonrheumatic heart valve disease. *Cardiovasc Pathol* 2007; 16:171-178.
- [47] Parolari A, Loardi C, Mussoni L et al. Nonrheumatic calcific aortic stenosis: an overview from basic science to pharmacological prevention. *Eur J Cardiothor Surg* 2009; 35:493-504.
- [48] Olsen LH, Mortensen K, Martinussen T, Larsson I, Baandrup U, Pedersen HD. Increased NADPH-diaphorase activity in canine myxomatous mitral valve leaflets. *J Comp Pathol* 2003; 129:120-130.
- [49] Pedersen HD, Schutt T, Sondergaard R, Qvortrup K, Olsen LH, Kristensen AT. Decreased plasma concentration of nitric oxide metabolites in dogs with untreated mitral regurgitation. *J Vet Intern Med* 2003; 17:178-184.

- [50] Moesgaard SG, Olsen LH, Viuff BM et al. Increased nitric oxide release and expression of endothelial and inducible nitric oxide synthases in mildly changed porcine mitral valve leaflets. *J Heart Valve Dis* 2007; 16:67-75.
- [51] Mow T, Pedersen HD. Increased endothelin-receptor density in myxomatous canine mitral valve leaflets. *J Cardiovas Ph* 1999;34:254-260
- [52] Rabus M, Demirbag R, Sezen Y et al. Plasma and tissue oxidative stress index in patients with rheumatic and degenerative heart valve disease. *Arch Turk Soc Cardiol* 2008; 36:536-540.
- [53] Tamura K, Fukuda Y, Ishizaki M, Masuda Y, Yamanaka N, Ferrans VJ. Abnormalities in elastic fibers and other connective-tissue components of floppy mitral valve. *Am Heart J* 1995; 129:1149-1158.
- [54] Ferrans VJ, Renteria VG, Jones M, Knight PO, Roberts WC. Ultrastructure of prolapsed (floppy) mitral valves (Abstract). *J Mol Cell Cardiol* 1977; 9(Suppl):22.
- [55] Baker PB, Bansal G, Boudoulas H, Kolibash AJ, Kilman J, Wooley CF. Floppy mitral valve chordae tendineae. Histopatologic alterations. *Hum Pathol* 1988; 19:507-512.
- [56] Caulfield JB, Page DL, Kastor JA, Sanders CA. Connective tissue abnormalities in spontaneous rupture of chordae tendineae. *Arch Pathol* 1971; 91:537-541.
- [57] King BD, Clark HA, Baba N, Kilman JW, Wooley CF. "Myxomatous" mitral valves: collagen dissolution as the primary defect. *Circulation* 1982; 66:288-296.
- [58] Lis Y, Burleigh MC, Parker DJ, Child AH, Hogg J, Davies MJ. Biochemical characterization of individual normal, floppy and rheumatic human mitral valves. *Biochem J* 1987; 244:597-603.
- [59] Rabkin E, Aikawa M, Stone J, Fukumoto Y, Libby P, Schoen F. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodelling in myxomatous heart valves. *Circulation* 2001; 104:2525-2532.
- [60] Kimura N, Shukunami C, Hakuno D et al. Local tenomodulin absence, angiogenesis and matrix metalloproteinase activation are associated with the rupture of the chordae tendinae cordis. *Circulation* 2008; 118:1737-1747.
- [61] Anné W, Willems R, Roskams T et al. Matrix metalloproteinases and atrial remodelling in patients with mitral valve disease and atrial fibrillation. *Cardiovasc Res* 2005; 67:655-666.
- [62] Han RI, Black A, Culshaw GJ, French AT, Else RW, Corcoran BM. Distribution of myofibroblasts, smooth-like cells, macrophages and mast cells in mitral valve leaflets of dogs with myxomatous mitral valve disease. *Am J Vet Res* 2008; 69:763-769.
- [63] Barth P, Koster H, Moosdorf R. CD34+ fibrocytes in normal mitral valves and myxomatous mitral valve degeneration. *Path Res Pract* 2005; 201:301-304.
- [64] Disatian S, Ehrhart EJ, Zimmerman S, Orton EC. Interstitial cells from dogs with naturally occurring myxomatous mitral valve disease undergo phenotype transformation. *J Heart Valve Dis* 2008; 17:402-411.

- [65] Ng C, Cheng A, Myers L et al. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest* 2004; 114:1586-1592.
- [66] Caira F, Stock S, Gleason T et al. Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. *JACC* 2006; 47:1707-1712.
- [67] Pedersen HD, Koch J, Poulsen K, Jensen AL, Flagstad A. Activation of the renin-angiotensin system in dogs with asymptomatic and mildly symptomatic mitral valvular insufficiency. *J Vet Intern Med* 1995; 9:328-331.
- [68] Pedersen HD. Effects of mitral valve insufficiency, sodium intake and place of blood sampling on the renin-angiotensin system in dogs. *Acta Vet Scand* 1996; 37:109-118.
- [69] Pedersen HD, Mow T. Hypomagnesemia and mitral valve prolapse in Cavalier King Charles spaniels. *Zentralbl Veterinarmed* 1998; 45:607-614.
- [70] Zdrojewski TR, Wyrzykowski B, Krupa-Wojciechowska B. Renin-aldosterone regulation during upright posture in young men with mitral valve prolapse syndrome. *J Heart Valve Dis* 1995; 4:236-241.
- [71] Di Salvo G, Pergola V, Ratti G et al. Atrial natriuretic factor and mitral valve prolapse syndrome. *Minerva Cardioangiol* 2001;49:317-325.
- [72] Gaffney FA, Karlsson ES, Campbell W et al. Autonomic dysfunction in women with mitral valve prolapse syndrome. *Circulation* 1979; 59:894-901.
- [73] Weissman NJ, Shear MK, Kramer-Fox R, Devereux RB. Contrasting patterns of autonomic dysfunction in patients with mitral valve prolapse and panic attacks. *Am J Med* 1987; 82:880-888.
- [74] Pasternac A, Kouz S, Gutkowska J et al. Atrial natriuretic factor: a possible link between left atrium, plasma volume, adrenergic control and renin-aldosterone in the mitral valve prolapse syndrome. *J Hypertens Suppl* 1986; 4:S76-79.
- [75] Lichodziejewska B, Klos J, Rezler J et al. Clinical symptoms of mitral valve prolapse are related to hypomagnesemia and attenuated by magnesium supplementation. *Am J Cardiol* 1997; 79:768-772.
- [76] Coghlan HC, Natello G. Erythrocyte magnesium in symptomatic patients with primary mitral valve prolapse: relationship to symptoms, mitral leaflet thickness, joint hypermobility and autonomic regulation. *Magnes Trace Elem* 1991-1992; 10:205-214.
- [77] Coats AJ. Ethical authorship and publishing. *Int J Cardiol* 2009; 131:149-50.

## Part II



## **4. Oxidative stress and valve disease: a new actor in the field?**

### **4.1 Oxidative stress and nitric oxide pathways in adult cardiac surgery patients: a comparative study**

#### **INTRODUCTION**

Oxidative stress represents an imbalance between the generation of reactive oxygen species and the ability of cells and tissues to readily detoxify them leading to organ damage and dysfunction. Although the role of oxidative stress and its relation to nitric oxide pathways derangements in cardiovascular diseases such as atherosclerosis, coronary artery disease, diabetes and heart failure is well known [1-3], much less information are available concerning patients undergoing adult cardiac surgery [4], especially for patients with heart valve disease. Also, previous studies on patients affected by valve disease have been much more focused on the presence of imbalances in valve tissue and not in the circulating levels of markers of oxidative stress and of nitric oxide pathways derangement [5, 6]; in other words, little is known concerning oxidative stress and nitric oxide pathways in patients who are undergoing adult cardiac surgery, especially concerning possible differences that could be related to different diseases (e.g. coronary vs. valve diseases). The aim of this study was then to evaluate oxidative stress and nitric oxide pathways markers in patients scheduled for isolated coronary bypass surgery, aortic valve replacement for calcific nonrheumatic aortic stenosis, and mitral valve repair for degenerative

mitral insufficiency, that are the three most common adult cardiac surgical procedures performed in adults of western countries, and to ascertain whether there are differences with healthy subjects and within the different patient populations.

## **PATIENTS AND METHODS**

### **Patients**

One hundred sixty-five consecutive patients candidates to undergo an elective, isolated adult cardiac surgical procedure (coronary artery bypass (CABG, n=63), aortic valve replacement for calcific nonrheumatic aortic stenosis (Aortic, n=51), mitral valve repair for degenerative mitral valve regurgitation (Mitral, n=51)) referred to our hospital were enrolled over a six-months time period (January 2011 – May 2011). Valve lesions were classified on the basis of echocardiographic and surgical findings.

Healthy subjects with cardiovascular risk factors (Controls, n = 33) were enrolled from those attending the clinic for global control of cardiovascular risk at Centro Cardiologico Monzino IRCCS. The clinical features of Controls, CABG, Aortic and Mitral patients are reported in Table 1.

All patients were assessed with detailed medical history, physical examination, EKG and echocardiography before scheduled surgery; for patients candidates to surgery, blood and urine collections were performed within 1 to seven days before surgery, and before coronary angiography, whereas Controls underwent samples collection at a scheduled follow-up visit.

Informed consent to participate to this observational study, which was approved by CCM Institutional Review Board, was obtained by all patients.

### **Oxidative stress and nitric oxide pathways markers.**

In this study we have assessed the following markers:

**Oxidative stress:** whole blood reduced glutathione(GSH)/disulphide glutathione(GSSG) ratio (GSH/GSSG); urinary 8-iso-prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>).

**Antioxidants:** plasma vitamin E (α-tocopherol, γ-tocopherol), and whole blood GSH.

**Nitric oxide pathways:** plasma arginine (Arg), asymmetric dimethylarginine (ADMA), symmetrical dimethylarginine (SDMA), Arg/ADMA ratio, Arg/SDMA ratio.

### **Sample collection**

Whole blood: peripheral blood sample was drawn from patients and controls while fasting, into tubes containing EDTA (9.3 mM; Vacutainer Systems, Becton Dickinson, Franklin Lakes, NJ, USA) kept on ice and immediately precipitated with 10% trichloroacetic acid (Sigma-Aldrich, St Louis, MO, USA) in 1 mM EDTA solution. After centrifugation at 10,000xg for 10 min at 4°C, the supernatant was stored at -80°C until analysis.

Plasma: EDTA-anticoagulated blood was centrifuged at 3000 g for 10 min at 4°C within 30 min after being drawn. Plasma was separated and aliquots were stored at -80°C until analysed.

Urine: an overnight urine collection the night before surgery or the night before visit was carried out and samples stored at -80°C until analyzed.

### **GSH and GSSG**

Levels of GSH and GSSG were determined in whole blood by liquid chromatography – tandem mass spectrometry (LC-MS/MS) method. Prior to analysis samples were thawed and diluted 1:20 or 1:400 with 0.1% formic acid.

The separation of analytes was conducted on a Luna PFP analytical column (100x2.0 mm, 3 µm, Phenomenex, Torrance, CA, USA) eluted at 35°C under isocratic conditions at 2 µL/min by 1% methanol in ammonium formate 0.75 mM adjusted to pH 3.5 with formic acid. LC-MS/MS was performed by Accela HPLC system coupled with a triple quadrupole mass spectrometer TSQ Quantum Access (Thermo Fisher Scientific, San Jose, CA, USA) using electrospray ionization source and multiple reaction monitoring (MRM) in positive mode. The MRM for GSH ( $m/z$  308.1→ $m/z$  76.2 + 84.2 + 161.9) and GSSG ( $m/z$  613.2→ $m/z$  230.5 + 234.6 + 354.8) were performed with collision energy optimized for each compound. Data were obtained after comparison with calibration curves using GSH and GSSG pure standard solutions (Sigma-Aldrich, St. Louis, MO, USA). The intra- and inter-CVs % obtained with standard samples were <5% for the both the analytes considered. The limit of

detection of GSH was 0.031  $\mu\text{mol/L}$ ; it was 0.008  $\mu\text{mol/L}$  for GSSG. Levels of GSH and GSSG were corrected for hemoglobin and expressed as  $\mu\text{mol/g Hb}$  as glutathione is mainly contained in erythrocytes.

### **Isoprostane determination**

Urinary 8-iso-PGF $2\alpha$  was detected by LC-MS/MS method according to Cavalca et al. [7]

Urinary concentration of prostanoids was calculated from the area ratio of the ion peaks of the 8-iso-PGF $2\alpha$  over the respective deuterated standard (8-iso-PGF $2\alpha$ -d $_4$ ). The estimated values were corrected for the urinary creatinine levels and expressed as picogram per milligram of creatinine. Creatinine was measured by standard method in the clinical laboratory of Centro Cardiologico Monzino using the Jaffe reaction.

### **Vitamin E**

Plasma  $\alpha$ - and  $\gamma$ -tocopherol were measured by HPLC (ESA Biosciences, Chelmsford, MA, USA) equipped with fluorimetric detector FP-1520 (Jasco, Tokyo, Japan), after organic extraction as previously described [8].

### **NO pathways**

Simultaneous determination of plasma Arginine, ADMA and SDMA was performed by LC-MS/MS as previously described [9].

## Statistical analysis

Continuous variables are summarized as mean  $\pm$  95% confidence intervals (CIs) if normally distributed; in case they were not normally distributed, they were Log-transformed before analysis. Categorical variables are summarized as frequency and percentage, and compared among groups by chi-square or Fisher exact test, as appropriate. Plasma biomarkers of oxidative balance and of NO pathway were firstly compared between Controls and patients candidate to surgery (CABG, Aortic, Mitral groups together) by general linear models (GLM). Three models were employed, with different levels of adjustment: model 1, unadjusted; model 2, adjusted for age and gender; model 3, adjusted for age, gender, BMI, hyperglycaemia, dyslipidemia, hypertension and treatment with anti-platelets, anti-hypertensive, nitrates and statins. The same models were employed to test the differences within the three groups of patients candidate to surgery. All the variables which yielded a significant p-value for ANOVA (three-group comparison) underwent a post-hoc analysis to test individual between-group differences.

We then analyzed the overall ‘pattern’ of oxidative balance and of NO pathway markers by including all ten variables (Table 2 and 3) in a principal component analysis (PCA). PCA is a statistical dimension-reduction technique widely employed to ‘compress’ a large sets of variables in few components (linear functions of the variables) which convey a large proportion of the information contained in the original data. We used the first two principal components to

plot the reciprocal distances of the four groups of subjects, in terms of oxidative balance and of NO pathway markers, in a bi-dimensional graph.

All tests were two-sided and p values <0.05 were considered as significant. All analyses were performed by SAS v. 9.2.

## RESULTS

**Patients.** As expected, patients groups were sensibly different in terms of preoperative clinical features (Table 1). This reflects the different natural history of the coronary, aortic and mitral valve diseases. Briefly, Mitral patients were the youngest whereas aortic patients were the oldest; CABG patients, were more frequently male gender, hypertensive and dyslipidemic. As expected, also drug regimens were sensibly different in patient populations, CABG patients receiving more frequently, statins, antiplatelet and anti-hypertensive drugs, followed by Aortic group.

**Oxidative stress.** Oxidative stress was significantly higher in patients candidate to surgery with respect to Controls (Table 2): the ratio between reduced and oxidized glutathione was higher in Controls, whereas urinary isoprostanes were lower. The analysis of the behavior of these markers in surgical candidates (Table 3) showed no differences among groups in urinary isoprostanes; on the other hand, GSH/GSSG ratio was lower in Mitral versus both CABG and Aortic groups, suggesting increased impairment of the glutathione pathway in these patients. Figure 1 summarizes the behavior of these markers.

**Antioxidants.** Plasma  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and GSH/Hb were higher in Controls with respect to patients scheduled for surgery (Table 2); no differences were detected in antioxidant markers related to the type of surgery (Table 3). Figure 2 outlines the antioxidant features in the different groups.

**Nitric oxide pathways.** Arginine levels, as well as SDMA levels and the ratio between arginine and SDMA did not differ between Controls and Patients; on the other hand, ADMA levels were increased in candidates to surgery, and there was also a trend towards a lower ratio between arginine and ADMA in these patients (Table 2). Interestingly, both Arg/ADMA and Arg/SDMA ratio were lower in Mitral patients with respect to CABG and Aortic groups, suggesting some degree of impairment of nitric oxide pathways for these patients (Table 3). Figure 3 reports the behavior of ADMA and of Arg/ADMA and Arg/SDMA ratios.

**Principal component analysis.** Figure 4 shows the location of the four groups in the plain defined by the two first principal components. The first principal component is positively correlated with antioxidant defences (mainly GSH) and with arginine, and negatively correlated with GSSG and ADMA; the second component is also positively correlated with antioxidant defences (mainly Vit. E), but is negatively correlated with arginine and positively with SDMA.



## DISCUSSION

In recent years basic and clinical research has underscored the main role of oxidative stress in cardiovascular disease. Enhanced production or attenuated degradation of reactive oxygen species affect endothelial and vascular function, and may contribute to atherosclerosis progression. Nitric oxide, released by normal endothelium, is one of the main determinants of normal endothelial and vascular function. It has been shown that cardiovascular risk factors can promote an imbalance between endogenous oxidants and antioxidants resulting in oxidative stress, impaired nitric oxide pathways function and, eventually, vascular dysfunction. The combination of oxidative stress and nitric oxide pathways imbalances may ultimately contribute to clinical cardiovascular events [10]. Previous studies have widely addressed the problem on oxidative stress in atherosclerosis and in coronary artery disease, suggesting that oxidative stress could even be considered as an unifying mechanism for many cardiovascular risk factors [11], and that a vicious circle between oxidative stress and inflammation can occur not only in the diseased arterial wall, where it causes loss of anti-oxidant protection and cell death, but also in adipose tissues, impairing adipocyte maturation and ultimately favoring insulin resistance [12].

Much less is known concerning the role of oxidative stress and of nitric oxide pathways in valve diseases; concerning the most frequent valve disease eventually leading to surgery, nonrheumatic calcific aortic stenosis, several studies have addressed the role of localized (within the diseased aortic valve

itself) oxidative stress documenting that it is increased in calcified areas of stenotic aortic valve [13], reactive oxygen species favor aortic valve calcification [5], and that uncoupled nitric oxide synthase activity may also contribute to and exacerbate oxidative stress [13]. But very limited evidence is available on systemic, circulating markers of both oxidative stress and nitric oxide pathways derangements. Ochoa and coll. studied 11 candidates to coronary bypass and aortic valve surgery showing that plasma levels of thiobarbituric acid reactive substances,  $\alpha$ -tocopherol, coenzyme Q and retinol were similar [14]; more recently, Ferrari and coworkers showed similar levels of ADMA and of homocysteine in healthy controls and in patients with calcific aortic stenosis scheduled for surgery [15].

If we consider the second most frequent valve disease of the adult, mitral valve regurgitation, current evidence is even more limited; only one paper dating back to 1985 has documented increased malondialdehyde levels in myxomatous mitral disease at echocardiography [16], and few animal studies have documented that a certain degree of oxidative stress may occur, both at a systemic and at a local level [17].

Our study was then designed to answer two main questions: a) first, do patients who are ready and need surgery for coronary, aortic and mitral disease differ with respect to control patients with cardiovascular risk factors in terms of oxidative stress and nitric oxide impairment?; b) second, are there differences related to the three main adult cardiac surgical pathologies? We selected all

comers for an elective isolated surgical procedure (associated procedures such as CABG plus valve were not enrolled) to try to give the most precise picture of the different diseases.

Data from our study clearly show that patients who are candidates to the most frequent cardiac surgical procedures of the adult have increased oxidative stress markers and reduced antioxidant levels with respect to controls, and this is paralleled by an increase of ADMA levels that suggest an impairment/interference in the production and release of nitric oxide. In other words, patients who require prompt cardiac surgery have increased impairment of these pathways with respect to patients who have cardiovascular risk factors but who are not candidates to surgery nor they had previous major cardiovascular events.

Second, there are some clear differences between the surgical pathologies, but also some similarities; interestingly, no differences at all could be documented in any of the studied variables concerning CABG and Aortic groups, either for the analysis of data without any adjustment or after adjustment for baseline differences in patient populations. This suggests that it is possible that the similarities between coronary disease and aortic valve disease may predominate on dissimilarities [18], determining a similar impairment of these pathways whose role in cardiovascular disease progression is still probably overlooked. On the other hand, the assessment of the behavior of the markers in Mitral patients suggests that these patients have both more marked oxidative stress with

respect to CABG and Aortic groups, and –especially- a more pronounced impairment of nitric oxide generation, as suggested by lower Arg/ADMA and Arg/SDMA ratios, suggesting a more marked unbalance favoring inhibitors of nitric oxide production and, as a consequence, a potentially higher degree of endothelial dysfunction. This is an unexpected finding, as the baseline clinical features of Mitral patients, who were younger (notably, their mean age was comparable to that of Controls) and had lower incidence of cardiovascular risk factors with respect to CABG and aortic groups, did not anticipate a possible worse scenario for these patients, at least in terms of oxidative stress and of nitric oxide. And these differences persisted even after the adjustment for baseline clinical features, confirming the robustness of these data. A possible explanation of this finding is currently lacking; we might speculate that it is possible that some differences in shear stress or in rheological features inherent to mitral valve regurgitation may contribute to that, as it is known that patients with mitral regurgitation show very striking perturbations of regurgitant flow in left atrium [19]; but this remains actually only a mere hypothesis.

This study has one main limitation, that there are several differences in baseline clinical features between the different patient populations. These differences were expected when designing this pilot study, but were accepted in order to gather the most reliable information concerning patients who need cardiac surgery for the three most frequent cardiac diseases of the adult: coronaries, aortic and mitral valves. And we choose to study all comers (with subsequent

analyses performed in order to adjust for different clinical features) instead of to perform a “cherry pick” among candidates.

In conclusion, our study shows that oxidative stress is increased in surgical candidates with respect to controls, and that Mitral patients have increased levels of this stress. In addition, Mitral patients have also a more pronounced impairment in nitric oxide generation. The molecular mechanisms underlying these findings as well as the possible clinical consequences have to be addressed in future studies.

**Table 1**

Preoperative clinical features of the study populations

Variable	Controls (n=33)	CABG (n=63)	Aortic (n=51)	Mitral (n=51)	pvalue
Age (years)	59 (55.2,62.8)	64 (62.0,66.0)	68 (66.1,69.9)	60 (57.0,63.0)	<b>&lt;0.001</b>
Male gender	22 (66.7%)	60 (95.2%)	29 (56.9%)	32 (62.7%)	<b>&lt;0.001</b>
Height (cm)	168 (165,171)	172 (170,174)	167 (165,169)	172 (169,175)	manca
Weight (kg)	77 (71.9,82.1)	80 (77.0,83.0)	74 (70.2,77.8)	74 (70.4,77.6)	<b>0.034</b>
Diabetes (%)	3 (9.1%)	16 (25.4%)	10 (19.6%)	5 (9.8%)	0.21
Diet-controlled	0	1 (1.6%)	1 (2.0%)	0	
Non-insulin dependent	3 (9.1%)	14 (22.2%)	9 (17.6%)	4 (7.8%)	
Insulin-dependent	0	1 (1.6%)	0	1 (2.0%)	
Hypertension (%)	16 (48.5%)	43 (68.3%)	31 (60.8%)	18 (35.3%)	<b>0.007</b>
Dyslipidemia (%)	16 (48.5%)	46 (73%)	28 (54.9%)	28 (54.9%)	<b>0.042</b>
Triglycerides (mg/dL)	113 (97.3,129)	131 (110,152)	104 (87.8,120)	98 (86.7,109.3)	0.09
HDL-cholesterol (mg/dL)	55 (49.5,60.5)	43 (40.8,45.2)	53 (49.2,56.8)	52 (48.2,55.8)	<b>&lt;0.0001</b>
LDL-cholesterol (mg/dL)	139 (127,151)	114 (105,123)	121 (112,130)	137 (126,148)	<b>0.007</b>
Total cholesterol (mg/dL)	216 (203,229)	182 (172,192)	196 (186,206)	210 (197,223)	<b>0.0006</b>
Preoperative creatinine (mg/dL)	0.83	0.93	0.86	0.88	0.09
Preoperative glucose (mg/dL)	106 (98.8,113)	121 (112,130)	115 (108,122)	107 (102,112)	<b>0.015</b>
Current smoker (%)	6 (18.2%)	13 (20.6%)	3 (5.9%)	7 (13.7%)	<b>0.011</b>
Echocardiographic EF (%)	62 (58.2,65.8)	59 (56.5,61.5)	60 (57.5,62.5)	63 (60.3,65.7)	0.07
Previous stroke (%)	0	2 (3.3%)	0	2 (3.9%)	0.38
Previous TIA (%)	0	3 (4.8%)	1 (2.0%)	1 (2.0%)	0.54
Drug therapy					
Antiplatelets (%)	2 (6.1%)	43 (68.3%)	19 (37.3%)	5 (9.8%)	<b>&lt;0.0001</b>
Angiotensin receptor blockers (%)	4 (12.1%)	11 (17.5%)	16 (31.4%)	5 (9.8%)	<b>0.001</b>
Converting enzyme inhibitors (%)	6 (18.2%)	22 (34.9%)	11 (21.6%)	18 (35.3%)	0.15
Beta-blockers (%)	2 (6.1%)	41 (65.1%)	17 (33.3%)	21 (41.2%)	<b>&lt;0.0001</b>
Calcium channel blockers (%)	3 (9.1%)	19 (30.2%)	14 (27.5%)	2 (3.9%)	<b>0.001</b>
Nitrates (%)		19 (30.2%)	2 (3.9%)	2 (3.9%)	<b>&lt;0.0001</b>
Statins (%)	6 (18.2%)	42 (66.7%)	17 (33.3%)	9 (17.6%)	<b>&lt;0.0001</b>

**Table 2**  
Oxidative stress markers. antioxidants and nitric oxide pathways.  
Controls vs. Patients candidates to surgery

Variable	Model	Controls (n=33)	Patients (n=165)	pvalue
<b>Log GSH/GSSG ratio</b>	1	3.19 (2.95. 3.43)	2.70 (2.60. 2.80)	<b>&lt;.001</b>
	2	3.19 (2.95. 3.43)	2.70 (2.60. 2.80)	<b>&lt;.001</b>
	3	3.35 (3.09. 3.61)	2.68 (2.58. 2.78)	<b>&lt;.001</b>
<b>Log 8-isoPGF2<math>\alpha</math></b> (pg/mg creatinine)	1	4.79 (4.63. 4.95)	5.18 (5.10. 5.26)	<b>&lt;.001</b>
	2	4.80 (4.64. 4.96)	5.17 (5.09. 5.25)	<b>&lt;.001</b>
	3	4.80 (4.62. 4.98)	5.18 (5.10. 5.26)	<b>&lt;.001</b>
<b><math>\alpha</math>-Tocopherol</b> ( $\mu$ g/mL)	1	14.1 (13.1. 15.0)	11.9 (11.5. 12.3)	<b>&lt;.001</b>
	2	13.9 (13.0. 14.9)	11.9 (11.5. 12.4)	<b>&lt;.001</b>
	3	13.5 (12.5. 14.5)	12.1 (11.6. 12.4)	<b>0.0068</b>
<b>Log <math>\gamma</math>-Tocopherol</b> (pg/mL)	1	2.45 (2.33. 2.57)	2.03 (1.97. 2.09)	<b>&lt;.001</b>
	2	2.43 (2.31. 2.55)	2.04 (1.98. 2.10)	<b>&lt;.001</b>
	3	2.40 (2.26. 2.54)	2.04 (1.98. 2.10)	<b>&lt;.001</b>
<b>GSH/Hb</b> ( $\mu$ mol/g Hb)	1	7.59 (7.29. 7.89)	6.96 (6.66. 7.26)	0.10
	2	7.62 (6.90. 8.34)	6.95 (6.65. 7.25)	0.090
	3	7.98 (7.22. 8.74)	6.90 (6.60. 7.20)	<b>0.011</b>
<b>Arginine</b> ( $\mu$ mol/L)	1	84.4 (75.7. 93.1)	83.5 (79.2. 87.9)	0.86
	2	82.3 (72.6. 91.9)	84.1 (79.6. 88.5)	0.75
	3	81.7 (70.4. 93.0)	84.1 (79.5. 88.7)	0.72
<b>ADMA</b> ( $\mu$ mol/L)	1	0.43 (0.39. 0.47)	0.49 (0.4. 0.51)	<b>0.010</b>
	2	0.43 (0.39. 0.47)	0.49 (0.47. 0.51)	<b>0.018</b>
	3	0.41 (0.37. 0.45)	0.49 (0.47. 0.51)	<b>0.0076</b>
<b>SDMA</b> ( $\mu$ mol/L)	1	0.38 (0.34. 0.42)	0.41 (0.39. 0.43)	0.33
	2	0.41 (0.37. 0.45)	0.40 (0.38. 0.42)	0.70
	3	0.42 (0.36. 0.48)	0.40 (0.38. 0.42)	0.55
<b>Arg/ADMA ratio</b>	1	202 (181. 223)	175 (165. 186)	<b>0.024</b>
	2	197 (174. 221)	176 (166. 187)	0.12
	3	202 (176. 228)	175 (165. 186)	0.075
<b>Arg/SDMA ratio</b>	1	230 (200. 260)	215 (200. 230)	0.37
	2	212 (180. 245)	219 (204. 234)	0.70
	3	210 (173. 248)	219 (204. 234)	0.68

**Table 3**  
Oxidative stress markers. antioxidants and nitric oxide pathways.  
Surgical candidates (CABG vs. Aortic vs. Mitral)

Variable	Model	CABG (n=63)	Aortic (n=51)	Mitral (n=51)	p-value
<b>Log GSH/GSSG ratio</b>	1	2.84 (2.68.3.00) <sup>##</sup>	2.73 (2.55.2.91)	2.51 (2.33.2.69)	<b>0.026</b>
	2	2.83 (2.65.3.01) <sup>#</sup>	2.74 (2.54.2.94)	2.51 (2.31.2.71)	0.058
	3	2.87 (2.67.3.07) <sup>###</sup>	2.80 (2.60.3.00) <sup>###</sup>	2.42 (2.22.2.62)	<b>0.008</b>
<b>Log 8-isoPGF2<sub>2</sub></b> (pg/mg creatinine)	1	5.14 (5.02.5.26)	5.26 (5.12.5.40)	5.13 (4.99.5.27)	0.32
	2	5.18 (5.04.5.32)	5.21 (5.07.5.35)	5.13 (4.99.5.27)	0.73
	3	5.19 (5.03.5.35)	5.21 (5.05.5.37)	5.14 (4.96.5.32)	0.83
<b>α-Tocopherol</b> (μg/mL)	1	11.4 (10.7.12.0)	12.1 (11.4.12.9)	12.3 (11.5.13.1)	0.15
	2	11.5 (10.8.12.2)	12.1 (11.3.12.9)	12.1 (11.3.12.9)	0.42
	3	11.9 (11.0.12.7)	11.9 (11.1.12.7)	11.9 (11.1.12.8)	1.00
<b>Log α-Tocopherol</b> (pg/mL)	1	1.99 (1.91.2.07)	2.11 (2.01.2.21)	2.02 (1.92.2.12)	0.18
	2	2.01 (1.93.2.09)	2.10 (2.00.2.20)	2.01 (1.91.2.11)	0.34
	3	2.04 (1.94.2.14)	2.09 (1.99.2.19)	1.98 (1.86.2.10)	0.41
<b>GSH/Hb</b> (μmol/g Hb)	1	7.10 (6.58.7.62)	6.71 (6.13.7.29)	7.01 (6.45.7.57)	0.58
	2	7.15 (6.61.7.69)	6.56 (5.96.7.16)	7.10 (6.50.7.70)	0.30
	3	7.05 (6.41.7.69)	6.74 (6.10.7.38)	7.13 (6.47.7.79)	0.66
<b>Arginine</b> (μmol/L)	1	83.8 (76.7.90.9)	89.5 (80.6.98.3)	78.7 (71.0.86.5)	0.19
	2	86.5 (78.9.94.0)	87.9 (78.7.97.0)	76.7 (68.9.84.5)	0.10
	3	89.8 (80.2.99.5)	87.4 (77.8.97.0)	73.1 (63.4.82.8)	0.053
<b>ADMA</b> (μmol/L)	1	0.45 (0.43.0.47) <sup>##</sup>	0.50 (0.46.0.54)	0.52 (0.48.0.56)	<b>0.005</b>
	2	0.46 (0.42.0.50) <sup>###</sup>	0.49 (0.45.0.53)	0.52 (0.48.0.56)	<b>0.031</b>
	3	0.48 (0.44.0.52)	0.49 (0.45.0.53)	0.50 (0.46.0.54)	0.86
<b>SDMA</b> (μmol/L)	1	0.39 (0.37.0.41)	0.40 (0.36.0.44)	0.43 (0.39.0.47)	0.35
	2	0.38 (0.34.0.42) <sup>#</sup>	0.40 (0.36.0.44)	0.43 (0.39.0.47)	0.11
	3	0.39 (0.35.0.43)	0.40 (0.36.0.44)	0.43 (0.39.0.47)	0.46
<b>Arg/ADMA ratio</b>	1	190 (174.205) <sup>##</sup>	180 (160.200) <sup>#</sup>	154 (137.171)	<b>0.011</b>
	2	192 (175.208) <sup>##</sup>	182 (162.202) <sup>#</sup>	150 (133.167)	<b>0.003</b>
	3	192 (171.212) <sup>#</sup>	181 (161.202) <sup>#</sup>	151 (130.171)	<b>0.032</b>
<b>Arg/SDMA ratio</b>	1	225 (201.249)	231 (201.261) <sup>#</sup>	191 (165.217)	0.077
	2	235 (211.260) <sup>##</sup>	227 (197.257) <sup>#</sup>	182 (156.207)	<b>0.008</b>
	3	239 (207.270) <sup>#</sup>	226 (194.258) <sup>#</sup>	178 (147.210)	<b>0.036</b>

<sup>#</sup>=p < 0.05vs. Mitral

<sup>##</sup>=p < 0.01 vs. Mitral



## REFERENCES

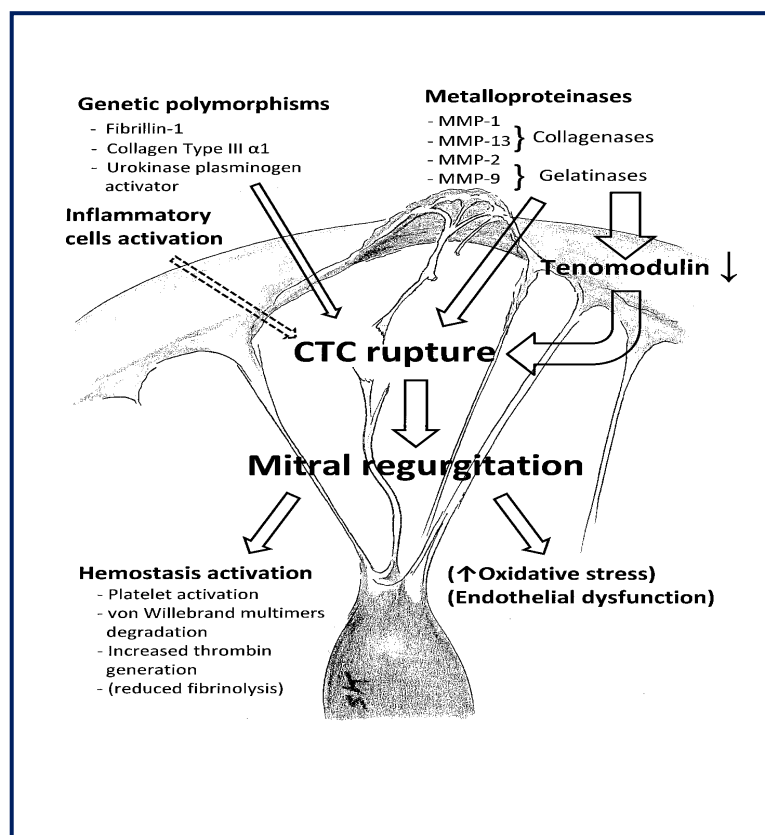
- [1] Scolletta S, Biagioli B. Energetic myocardial metabolism and oxidative stress: let's make them our friends in the fight against heart failure. *Biomed Pharmacother* 2010;64:203-7.
- [2] Visser M, Paulus WJ, Vermeulen MA, et al. The role of asymmetric dimethylarginine and arginine in the failing heart and its vasculature. *Eur J Heart Fail* 2010;12:1274-81.
- [3] Cavalca V, Cighetti G, Bamonti F, et al. Oxidative stress and homocysteine in coronary artery disease. *Clin Chem* 2001;47:887-92.
- [4] Cavalca V, Sisillo E, Veglia F, et al. Isoprostanes and oxidative stress in off-pump and on-pump coronary bypass surgery. *Ann Thorac Surg* 2006;81:562-7.
- [5] Liberman M, Bassi E, Martinatti MK, et al. Oxidant generation predominates around calcifying foci and enhances progression of aortic valve calcification. *Arterioscler Thromb Vasc Biol* 2008;28:463-70.
- [6] Rajamannan NM. Bicuspid aortic valve disease: the role of oxidative stress in Lrp5 bone formation. *Cardiovasc Pathol* 2011;20:168-76.
- [7] Cavalca V, Minardi F, Scurati S, et al. Simultaneous quantification of 8-isoprostaglandin-F(2alpha) and 11-dehydro thromboxane B(2) in human urine by liquid chromatography-tandem mass spectrometry. *Anal Biochem* 2010;397:168-74.
- [8] Werba JP, Cavalca V, Veglia F, et al. A new compound-specific pleiotropic effect of statins: modification of plasma gamma-tocopherol levels. *Atherosclerosis* 2007;193:229-33.
- [9] Squellerio I, Tremoli E, Cavalca V. Quantification of arginine and its metabolites in human erythrocytes using liquid chromatography-tandem mass spectrometry. *Anal Biochem* 2011;412:108-10.
- [10] Forstermann U. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. *Nat Clin Pract Cardiovasc Med* 2008;5:338-49.
- [11] De Rosa S, Cirillo P, Paglia A, Sasso L, Di Palma V, Chiariello M. Reactive oxygen species and antioxidants in the pathophysiology of cardiovascular disease: does the actual knowledge justify a clinical approach? *Curr Vasc Pharmacol* 2010;8:259-75.
- [12] Hulsmans M, Holvoet P. The vicious circle between oxidative stress and inflammation in atherosclerosis. *J Cell Mol Med* 2010;14:70-8.
- [13] Miller JD, Chu Y, Brooks RM, Richenbacher WE, Pena-Silva R, Heistad DD. Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. *J Am Coll Cardiol* 2008;52:843-50.
- [14] Ochoa JJ, Vilchez MJ, Mataix J, Ibanez-Quiles S, Palacios MA, Munoz-Hoyos A. Oxidative stress in patients undergoing cardiac surgery: comparative study of revascularization and valve replacement procedures. *J Surg Res* 2003;111:248-54.
- [15] Ferrari G, Sainger R, Beckmann E, et al. Validation of plasma biomarkers in degenerative calcific aortic stenosis. *J Surg Res* 2010;163:12-7.
- [16] Arocha F, Diez-Ewald M, Durango AI, Sulbaran T. Platelet activity in mitral valve prolapse: a study of platelet aggregation, malondialdehyde production, and plasma beta-thromboglobulin. *Am J Hematol* 1985;19:21-5.
- [17] Loardi C, Alamanni F, Trezzi M, et al. Biology of mitral valve prolapse: the harvest is big, but the workers are few. *Int J Cardiol* 2011;151:129-35.
- [18] Parolari A, Loardi C, Mussoni L, et al. Nonrheumatic calcific aortic stenosis: an overview from basic science to pharmacological prevention. *Eur J Cardiothorac Surg* 2009;35:493-504.
- [19] Dyverfeldt P, Kvitting JP, Carlhall CJ, et al. Hemodynamic aspects of mitral regurgitation assessed by generalized phase-contrast MRI. *J Magn Reson Imaging* 2011;33:58.

## 4.2 New surgical techniques and oxidative stress: is there any link possible ?

In the recent years all the community of cardiac surgeons reached the conviction that preserve as much tissue as possible (*"Respect rather than resect"* philosophy) is fundamental to achieve the best results in terms of morbidity, mortality and outcomes in the field of heart valve surgery.

Therefore, in the light of the fact that sparing the patient's own subvalvular apparatus the left ventricle is known to perform more efficiently, some cardiac surgeon are proposing new techniques that should improve the long term outcomes of the procedure.

Furthermore, the implication of the oxidative stress in the degeneration of the native heart valves and in the failure of the aortic and mitral valve repair is a field still under study that may have several practical implication.

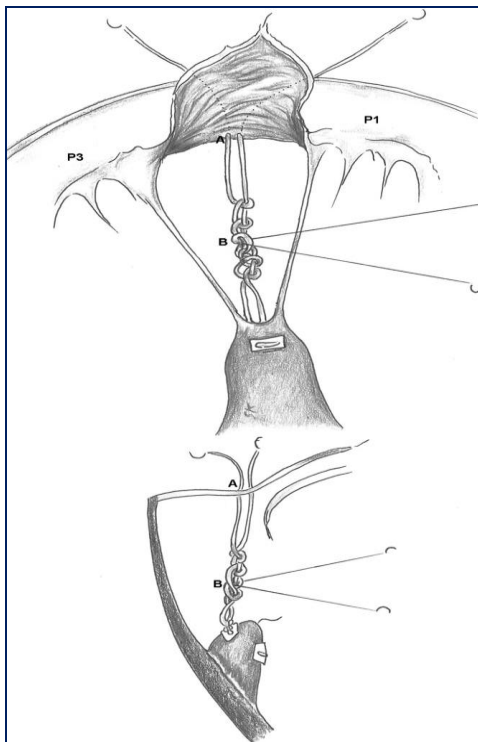


As illustrated in the above cartoon, the mechanic stress due to chordal rupture in mitral valve prolapse leads to the activation of several biometabolic pathways that, in the end, bring to an increased oxidative stress at cellular level and finally to endothelial dysfunction.

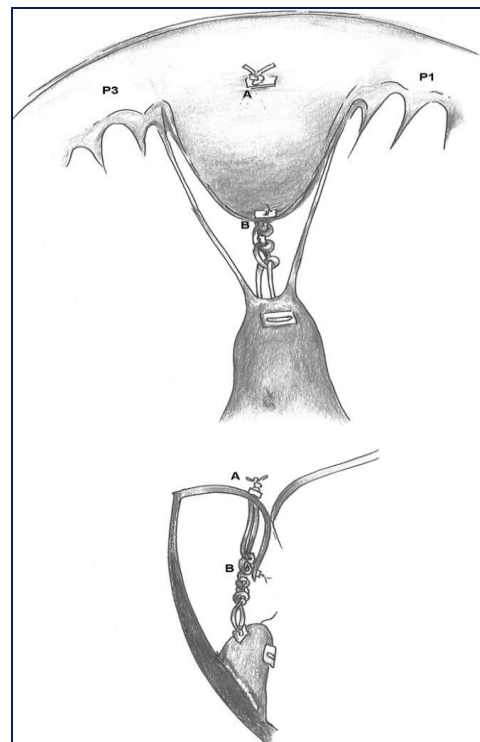
Similarly, the correction of the defect using surgical techniques that tend to stretch the leaflet and the papillary muscles may lead to the same oxidative stress activation and, in the end, to the failure of the valvuloplasty.

Personally, I have developed new techniques briefly described below that allowing more physiologic transvalvular flow should reduce the mechanic stress to the ventricle and finally optimize the surgical repair.

### ▪ Tent-shape technique: another procedure to repair P2 of posterior leaflet of mitral valve



**Fig. 1.** The new chord is attached to the suitable papillary muscle. Many loose reverse knots are tied (making small loops) so that the whole length of the knots reaches the mid expected length of the new chord. The two needles are passed through the body of P2 from the ventricular to the atrial side at the same height of non-prolapsing P1 and P3 of the posterior leaflet (point A). The anchor for the edge of the excess portion of P2 (point B) is determined on the new chord. One suture of 4/0 Cardionyl is inserted in the appropriate small loop.



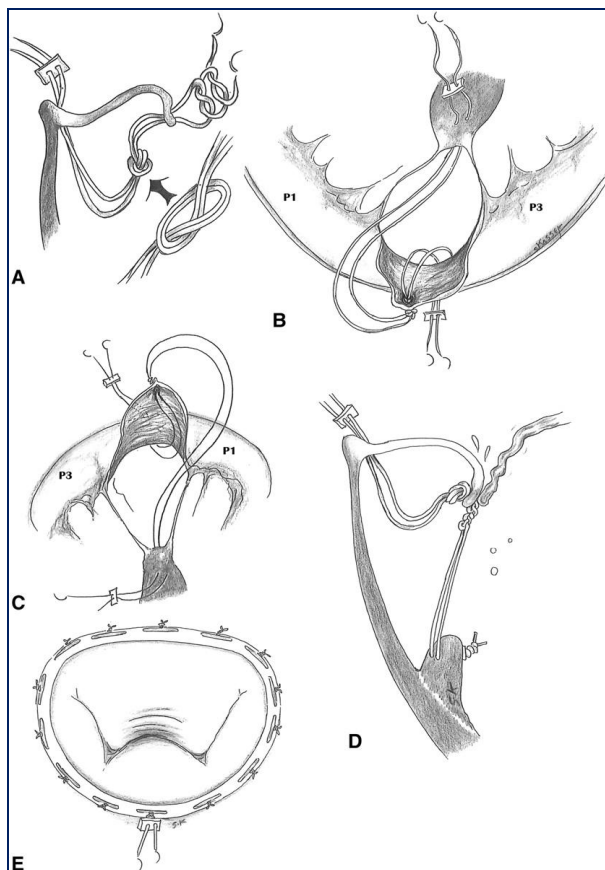
**Fig. 2.** The Gore-Tex suture is tied onto a strip of pericardium after an estimation of the chord length (point A). The two arms of the 4/0 Cardionyl suture are then passed through the thickened P2 free edge and knotted over a pledget of pericardium.

An accurate valve analysis is performed: the P2 height is assessed, the ruptured or redundant chords are identified, and papillary muscles are inspected to identify the suitable muscle for chordal attachment. To facilitate visualization of the subvalvular apparatus, the elongated chordae of P2 can sometimes be resected. Thereafter, the two needles of a 5/0 polytetrafluoroethylene (Gore-Tex, W.L. Gore & Associates, Newark, Delaware, USA) suture, supported by a felt pledget, are passed through the tip of the appropriate papillary muscle with a forehanded technique, so the new chord arises from the papillary side that faces the posterior ventricular wall. The Gore-Tex is tied loosely. Then, many loose reverse knots are tied (making small loops), as it is required that the whole length of the knots reaches the mid-expected length of the new chord. The length of second- or third-order chordae of the non-prolapsing portion of P2 or the non-prolapsing A2 of the anterior leaflet can be used as a reference point to measure the expected length of the new chord. The two needles are subsequently passed through the body of P2 from the ventricular to the atrial side at the same height of non-prolapsing P1 and P3 of the posterior leaflet (*point A, Fig. 1*). The suture is left untied. The appropriate length of the new chord is estimated by injection of saline solution into the left ventricular cavity with a considerable pressure. The Gore-Tex suture is then tied onto a strip of pericardium, maintaining both the edge of the anterior leaflet (A2) and the point A (the anchor for the new chord in the body of P2) at the same level. Next, the anchor for the edge of the excess portion of P2 (*point B, Fig. 1*) is determined on the new chord (one of the multiple small loops). The distance between the anchor for the edge of P2 (point B) and the anchor for the new chord on the leaflet (point A) should be equal to or less than the length of the excess portion of P2. In addition, in case of high risk of SAM, it is advisable that the anchoring for the P2 edge on the new chord should be toward the papillary muscle. One suture of 4/0 Cardionyl is inserted in the appropriate small loop (anchor for the edge of P2; point B, Fig. 1) and tightly tied. The two arms of the suture are then passed through the

thickened P2 free edge and knotted over a pledget of pericardium (*Fig. 2*). Valve competency is then checked by fluid testing. The repair procedure is completed by implantation of the annuloplasty ring of choice.

### ▪ **Annulus-edge-papillary stitch to repair P2 of the mitral valve posterior leaflet**

Transesophageal echocardiography is performed, and cardiopulmonary bypass is established. Antegrade and retrograde cold-blood cardioplegia, CO<sub>2</sub> insufflation of the operative field, and moderate hypothermia (32°C) are routinely used. The valve is inspected, and after confirmation of the prolapsing P2, a pair of 5-0 polytetrafluoroethylene (Gore-Tex, WL Gore & Associates, Inc, Newark, DE) sutures are passed through a pledget and the posterior MV annulus from the atrial to the ventricular side at the site corresponding to the tip of the prolapsing P2. A non-sliding knot is then tied on the ventricular side (Figure 1, A). The 2 arms of the sutures are passed through the thickened P2 free edge from the ventricular to the atrial side and tightly knotted on the atrial surface (Figure 1, A). Thereafter, the 2 needles are passed through the tip of the appropriate papillary muscle with a backhanded technique and then through an expanded polytetrafluoroethylene pledget without tying (Figure 1, B, C). Thus, the new chord has an obtuse angle formed by a vertex (the P2 edge ) and 2 rays: the edgepapillary (E-P suture) and edge-posterior annulus (E-A suture). By using the nonprolapsed anterior leaflet as a guide, the length of the E-P component is determined, taking into consideration that it should be shorter than the non-prolapsing chord to bring down the excessive portion of P2 into the ventricular cavity. A metal clip is placed across the 2 arms of the E-P suture ahead of the expanded polytetrafluoroethylene pledget. The valve is tested by injecting cold saline into the ventricular cavity, and the valvular competence is determined by the length of the E-P component. The 2 arms are then tied tightly against the clip, which is removed and cut (Figure 1, D).



**FIGURE 1.** **A,** Pair of 5-0 polytetrafluoroethylene (Gore-Tex; WL Gore & Associates, Inc, Newark, DE) sutures are passed through the posterior MV annulus from the atrial to the ventricular side at the site corresponding to the tip of the prolapsing P2. A non-sliding knot is tied on the ventricular side. The 2 arms of the sutures are passed through the thickened P2 free edge from the ventricular to the atrial side and tightly knotted to anchor the P2 edge into the new chord. **B,** The 2 needles are passed through the tip of the papillary muscle with a backhanded technique and then through an expanded polytetrafluoroethylene pledget without tying. Thus, the new chord arises from the papillary side that faces the posterior ventricular wall. **C,** AEP stitch as seen by the surgeon's assistant. **D,** Cross-profile shows that the length of the E-P component is determined by saline injection test. **E,** Slightly oversized ring is inserted into the mitral annulus with interrupted horizontal mattress sutures. The pledget of the untied atrial E-P suture behind the ring is free. Another saline injection test is performed with considerable pressure once the ring is in its definitive position. The valve is continent but anatomically abnormal (sadshape).

The annuloplasty ring is sized according to the height of the well-stretched anterior leaflet, and a slightly oversized ring is inserted into the mitral annulus with interrupted horizontal mattress sutures of 2-0 Polydek, being careful to keep free the pledget of the untied atrial E-P suture (annular chordal anchor) behind the ring's mid-posterior portion (Figure 1, E). Once the ring is in its definitive position, the valve is again tested by injecting cold saline with considerable pressure (Figure 1, E). The height of the P2 is controlled by the E-A component's length, so the untied atrial suture behind the ring is carefully pulled to decrease the height of P2 (Figure 2, A), allowing the A2 of the anterior leaflet to occupy its space on the radial direction of the valvular area until the MV regains its smiles shape and the first slight insufficiency appears, while both the left ventricle and leaflets are distended under the filling pressure (Figure 2, B). A metal clip is engaged across the 2 arms behind the mid-posterior portion of the ring. The 2 arms are then tied tightly against the clip and cut. The clip is

removed. If the P2 is high and wide with a notable shape of a square, rectangle, or trapezoid, more than 1 AEP stitch should be made with the same technique (Figure 2, C). In cases with a high risk of systolic anterior motion, for example, a small left ventricular cavity or redundant anterior leaflet, and before repairing the MV with the AEP stitch, the P2 can be remodeled by a small triangular-shaped resection with the apex toward the annulus (Figure 2, D, E). Since August 2010, we have applied the AEP stitch technique in 5 patients with severe MV regurgitation caused by isolated prolapse of a high P2 with excellent results.

- **Papillary muscle-to-anterior annulus stitches: Another technique to prevent systolic anterior motion after mitral valve repair**

The inspection of the mitral valve is carried out as usual. The leaflets are accurately analyzed, and the papillary muscles are inspected to identify the posterior head of each papillary muscle. A double-armed 4-0 polytetrafluoroethylene (Gore-Tex; W. L. Gore & Associates, Flagstaff, Ariz) suture supported by a felt pledget is passed twice through the tip of the posterior head of the posterior papillary muscle with a forehand technique and is fixed with a loose knot to avoid necrosis. The ventricular surface of the anterior leaflet is inspected with two nerve hooks to explore the attachment of secondary chord, which arises from the posterior papillary muscle (preferably the most distant from the leaflet's base, and toward the mitral anteroposterior axis). The height of the selected chord is used as a guide for determining the length of the first part of the new chord. A metal clip is placed across the 2 arms of the new chord at the level of the anchor of the stretched native chord selected. The trace of the attachment of the selected chord on the atrial surface of the anterior leaflet is determined by insertion of a thin insulin needle as a marker beside the attachment's site.



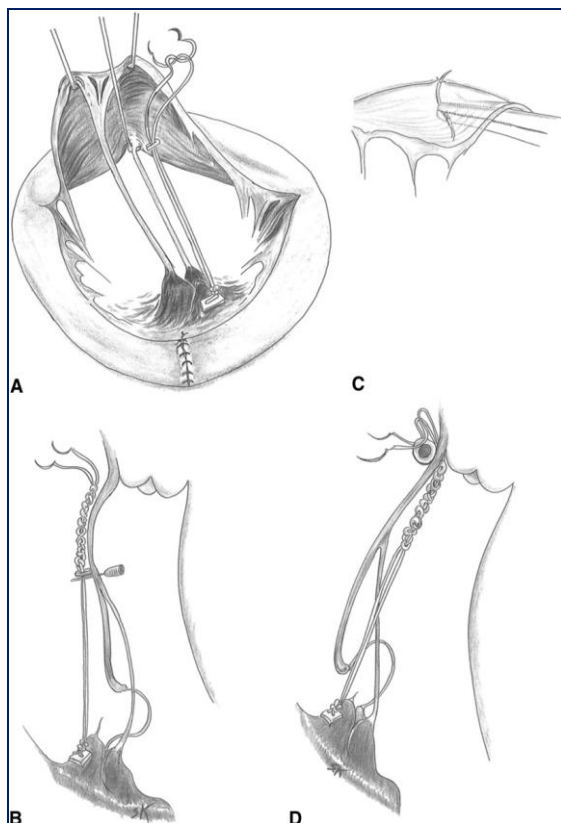
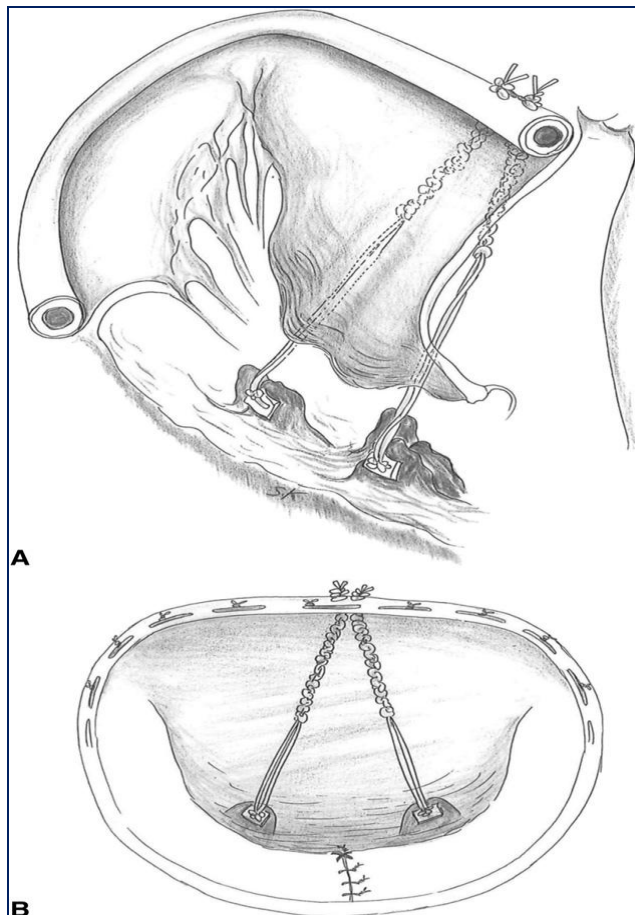


FIGURE 1. A, A double-armed 4-0 polytetrafluoroethylene (Gore-Tex;W. L. Gore & Associates, Flagstaff, Ariz) suture is passed twice through the tip of the posterior head of the posterior papillary muscle with a forehand technique. The new chord thus arises from the papillary side that faces the posterior ventricular wall. The surgeon's assistant uses a nerve hook to stretch the second chord selected. The length of the selected chord is used as a guide to determine the first part of the new chord. A metal clip is engaged across the 2 arms at the level of the attachment of the stretched native chord. B, Cross-profile illustration shows a thin insulin needle inserted from the ventricular to the atrial side through the anterior leaflet in the site of native chordal attachment. The distance from the marker to the anterior annulus determines the length of the second part of the new chord. Multiple knots have been tied against the metal clip. The whole length of the knots should correspond to the distance between the marker and midanterior mitral annulus, which is equal to the distance between the chordal attachment and the anterior annulus. C, The 2 arms of the suture are then passed through at about the midanterior mitral leaflet from the ventricular to the atrial surface side by means of a backhand technique, taking care not to injure the aortic valve. The surgeon thus cannot see the point of entry. D, Cross-profile Illustration represents the anterior ring in its definitive position. The marker has been removed. The needles of the atrial side of the Gore-Tex sutures are passed through the anterior prosthetic sheath.

Thereafter, the anterior leaflet is once again pulled down into the ventricular cavity, leaving the insulin needle in place. On the atrial surface of the anterior leaflet, the distance between the marker (the insulin needle) and the anterior annulus determines the length of the second part of the new chord, which will be anchored to the anterior annulus with no excessive tension during the presystolic phase. Multiple knots are then tied against the metal clip until the whole length of the knots corresponds to the distance between the marker and midanterior mitral annulus. Subsequently, the clip is removed. Thereafter the anterior leaflet is pulled up to maximum with a nerve hook, and the insulin needle is gently removed from the anterior leaflet. The 2 arms of the suture are then passed through approximately the middle of the anterior mitral leaflet from the ventricular to the atrial surface side by means of a backhand technique, and the suture is left untied.





**FIGURE 2.** **A,** Cross-profile illustration shows the second papillary muscle-to-anterior annulus stitch, which arises from the posterior head of the anterior papillary muscle. The 2 new chords are tightly tied onto the prosthetic sheath. **B,** Illustration of the final position of the 2 papillary muscle-to-anterior annulus stitches as they would be seen by the surgeon if the anterior leaflet were transparent.

The procedure is repeated step by step to implant the second papillary muscle–anterior annulus chord, which arises from the posterior head of the anterior papillary muscle and is anchored to about the middle of the anterior mitral annulus. Subsequently, the surgeon continues the mitral repair, choosing the appropriate strategies for the lesions of the valve identified during the valve analysis. In our 4 selected cases, the posterior leaflet repair was performed with a traditional triangular resection. Finally, an annuloplasty Physio ring (Physio-Control, Inc, Redmond, Wash) is sized and added to complete the repair, maintaining free the untied Gore-Tex sutures outside the ring. When the ring is in its final position, the Gore-Tex sutures are passed through the anterior prosthetic sheath and tightly knotted. After fluid testing to confirm the valve’s competence, the atrium is once again closed.

In conclusion we can affirm that new surgical skills and techniques are required in order to perform procedures that can lead to more physiological repair of the heart valves; this might have a clinical and practical implications.

Further studies are necessary to improve our knowledge about the role of oxidative stress in the outcome of cardiac valve repair.

## REFERENCES

- [1] Cohn LH, Couper GS, Aranki SF, Rizzo RJ, Kinchla NM, Collins JJ Jr. Long-term results of mitral reconstruction for regurgitation of the myxomatous mitral valve. *J Thorac Cardiovasc Surg.* 1994;107:143-51.
- [2] Jebara VA, Mihaileanu S, Acar C, Brizard C, Grare P, Latremouille C, et al. Left ventricular outflow tract obstruction after mitral valve repair. Results of the sliding leaflet technique. *Circulation.* 1993;88(5 Pt 2):II30-4.
- [3] Lee KS, Stewart WJ, Lever HM, Underwood PL, Cosgrove DM. Mechanism of outflow tract obstruction causing failed mitral valve repair. Anterior displacement of leaflet coaptation. *Circulation.* 1993;88(5 Pt 2):II24-9.
- [4] Mihaileanu S, Marino JP, Chauvaud S, Perier P, Forman J, Vissoat J, et al. Left ventricular outflow obstruction after mitral valve repair (Carpentier's technique). Proposed mechanisms of disease. *Circulation.* 1988;78(3 Pt 2):I78-84.
- [5] Ranganathan N, Lam JHC, Wigle ED, Silver MD. Morphology of the human mitral valve. II. The valve leaflets. *Circulation.* 1970;41:459-66.
- [6] Filsoufi F, Carpentier A. Reconstructive surgery in mitral valve disease. *Semin Thorac Cardiovasc Surg.* 2007;19:103-10.
- [7] Galloway AC, Colvin SB, Baumann FG, et al. A comparison of mitral valve reconstruction with mitral valve replacement: intermediate-term results. *Ann Thorac Surg.* 1989;47:655-62.
- [8] Cohn LH, DiSesa VJ, Couper GS, et al. Mitral valve repair for myxomatous degeneration and prolapse of the mitral valve. *J Thorac Cardiovasc Surg.* 1989;98:987-3.
- [9] Carpentier A. Reconstructive valvuloplasty. A new technique of mitral valvuloplasty. *Presse Med* 1969;77:251—3.
- [10] Mohty D, Orszulak TA, Schaff HV, Avierinos JF, Tajik JA, Enriquez-Sarano M. Very long-term survival and durability of mitral valve repair for mitral valve prolapse. *Circulation* 2001;104(Suppl. 1):I1—7.
- [11] Perier P, Stumpf J, Gotz C, Lakew F, Schneider A, Clausnizer B, Hacker R. Valve repair for mitral regurgitation caused by isolated prolapse of the posterior leaflet. *Ann Thorac Surg* 1997;64:445—50.
- [12] Adams DH, Anyanwu AC. Seeking a higher standard for degenerative mitral valve repair: begin with etiology. *J Thorac Cardiovasc Surg* 2008;136:551— 6.
- [13] Mihaljevic T, Blackstone EH, Lytle BW. Folding valvuloplasty without leaflet resection: simplified method for mitral valve repair. *Ann Thorac Surg* 2006;82:e46— 8.

## 5. Conclusions

In the last two decades we faced a huge changing in thinking about heart valve pathology and surgical treatment. The development of new knowledge about the cellular mechanisms of damage may let us understand the intrinsic pathophysiology of the degenerative process that leads to the aortic and mitral valve degeneration and lost of function. In particular, as described in this work, oxidative stress seems to play a central role in this degenerative process and so medical and surgical options that can improve the cellular and tissutal response to the free oxygen radicals are expected to ameliorate the clinical outcome.

From the practical point of view, the application of the knowledge arisen from the “translational research”, i.e. that branch of the research who could quickly applied in clinical practice, may leads to the progression of new and more efficient treatment’s modality.

The concepts of “*respect rather than resect*” and the development of new surgical techniques are emerging to put the surgical treatment of valve disease closer to the normal physiology of the cardiac valves.

More studies, in particular those analyzing the results of these new and more conservative techniques are required to confirm the promising initial results.

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